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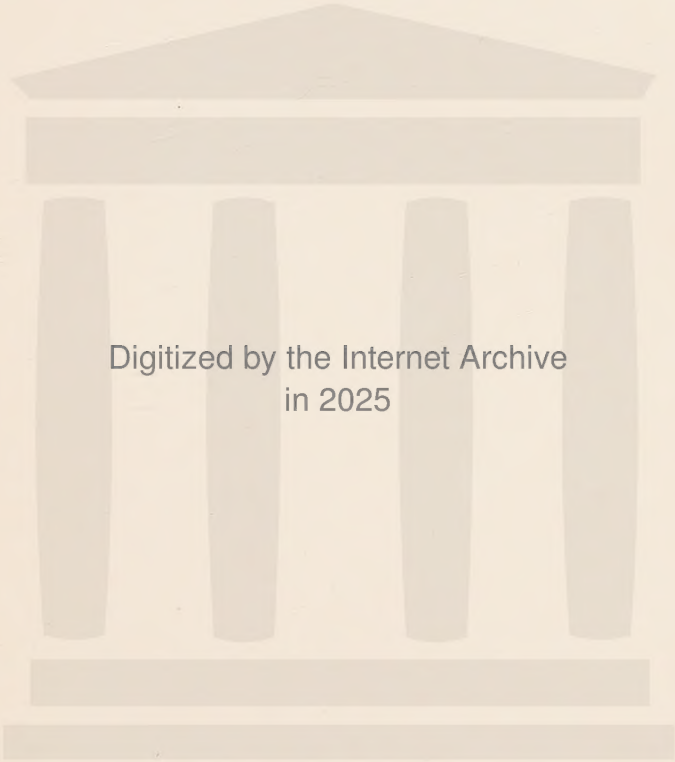
SPECTRUM ANALYSIS
APPLIED TO
BIOLOGY AND MEDICINE

C. A. MACMUNN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SPECTRUM ANALYSIS

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BIOLOGY AND MEDICINE

Yours sincerely
C. A. MacMunn.

SPECTRUM ANALYSIS

APPLIED TO

BIOLOGY AND MEDICINE

BY THE LATE

C. A. MACMUNN, M.A., M.D.

AUTHOR OF "THE SPECTROSCOPE IN MEDICINE," ETC., AND ARTICLES IN THE "ENCYCLOPÆDIA BRITANNICA" AND "QUAIN'S DICTIONARY OF MEDICINE"

WITH A PREFACE BY

F. W. GAMBLE

WITH ILLUSTRATIONS

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1914

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Dedication

TO

ONE WHO HELPED ME THROUGH DAYS OF TOIL AND TROUBLE

WHEN EVERYTHING SEEMED HOPELESS,

I, HER HUSBAND AND COMPANION,

DEDICATE THIS SMALL TRIBUTE OF AFFECTION.

NOTE.

I CANNOT allow this work of my late husband to go to press without expressing my deep and lasting gratitude to those who have helped me in this labour of love.

I wish in particular to thank Professor Gamble, who has from the first helped me with his advice and sympathy ; also Dr. Milroy of Belfast, who has shown such generous zeal in the revision of this book ; and finally to my husband's friend, Dr. C. A. Stidston, whose devotion to his memory has been untiring.

S. B. MACMUNN.

175 JEFFCOCK ROAD,
WOLVERHAMPTON, 18 *Feb.*, 1914.

PREFACE.

In the difficult field of spectrum analysis applied to the study of animal pigments, the researches of the late Dr. MacMunn hold a high place. Unlike many medical workers, Dr. MacMunn extended the scope of his observations so as to include the pigments of most Invertebrate as well as those of certain Vertebrate animals and of man : and, as the present volume shows, his inquiry also extended to the colouring matters of plants. Highly appreciated as his labours have been, perhaps more generally in Germany and America than at home, few of those who cultivate this field of knowledge are aware of the great difficulties under which he laboured. His research work was done in time saved from other and exacting professional employment, in a town far (at that time) from University life, with but scanty encouragement from or intercourse with other scientific workers. Such a feat argues talent of no ordinary order, devotion of unusual strength, and originality, exceptional both in conception and in execution.

Dr. MacMunn was born in Seafield, Co. Sligo, in 1852, and was trained at Trinity College, Dublin, where he graduated B.A. (honours) in 1871 and M.B.

in 1872. For some twenty years after qualifying, MacMunn was engaged in practice at Wolverhampton, and it was during this period that his chief scientific research was accomplished. Honorary Pathologist and Physician to the South Staffordshire General Hospital, and carrying on an onerous medical practice, MacMunn was nevertheless an ardent supporter of the Volunteer movement and devoted much time and energy to its advancement. In the last few years of his life he acted as Administrative Medical Officer of the North Midland division of the Territorial force. When the Boer war broke out, MacMunn volunteered for service and was appointed by Lord Roberts as Staff Officer to the Royal Hospital Commission. For these services he received notice in dispatches and the war medal with three clasps, but his health broke down, and his death in February 1911 was probably traceable to the effects of the strain of his life in South Africa. After his return MacMunn was made a county J.P. and a life Governor of Birmingham University.

His most distinctive characteristic was indefatigable devotion to scientific research. With the brilliant gifts which are so frequently found in Irishmen he combined a tenacity of purpose and capacity for achievement that are perhaps less frequently associated with them. His researches, conducted after his day's work was done, and with but little encouragement from the "doyens" of physiology, have given him an enduring name. His chief work appeared in publications of the Royal Society, of the Physiological Society and other Journals (a list of which is given in the bibliography of this

book); but his books "The Spectroscope in Medicine" and "Outlines of Clinical Chemistry" have been very widely accepted as authoritative expositions. But of all the subjects he touched upon and illuminated, the subject of pigments was the one which attracted him most and to the knowledge of which he contributed the most notably, both as regards improvement in technique and in the value and range of his results. No doubt the professional physiologist will find ground for criticism in regard to MacMunn's treatment of certain problems in Chromatology; but when his isolated position is considered, his scanty leisure, his want of Laboratory equipment, it is not the occasional incompleteness of treatment that we wonder at, but at the fact that under conditions that ordinarily occupy the whole life of a medical man, MacMunn not only fulfilled the duties of a Magistrate and a Colonel of the Territorial force, but found time and energy to publish original observations which have always to be reckoned with in the medical and biological study of pigments.

The present work was begun and continued between attacks of serious illness, and therefore lacks the revision which he intended to give. The work of revising and completing the MS. for press has been entrusted to Dr. J. H. Milroy, whilst Dr. C. A. Stidston has devoted himself to the correction of the proofs.

F. W. GAMBLE.

The University,
Birmingham, 7 *Feb.*, 1914.

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CHAPTER I.

THE PRISM.

The spectroscope is the instrument employed for the analysis of light into its components. As a result of its action these components appear as a bright band of colours, arranged side by side, which may or may not form a continuous series according to the nature of the light examined. In the earliest and best-known form of the spectroscope the separation of any sort of light into its constituents is brought about by means of a prism of glass, quartz, or other transparent material.

The prism used for spectroscopic work is a solid bounded by five surfaces, two of which are triangles, equal, similar, and parallel to each other, while the other three are rectangular. The two triangular surfaces form the top and bottom of the prism, and, when placed on the table of the spectroscope, the prism rests on one of them. Two of the rectangular surfaces are ground flat and highly polished. These meet in an edge called the refracting edge of the prism. The third rectangular surface is ground flat but left unpolished. The angle between the two polished surfaces is known as the refracting angle of the prism.

When a ray of light passes obliquely through a plate of glass, bounded by parallel surfaces, the emergent ray is found to have undergone a lateral displacement, but to have retained the same direction as the

incident, the angular deviation of the ray at the one surface being exactly counteracted by that at the second. On the other hand, when the two surfaces are not parallel but inclined at an angle, the angular deviation of the ray of light occurring at the first surface is increased, not counteracted on its emergence from the second surface.

The foregoing statements apply strictly only to rays of homogeneous light, that is, light of one colour or wave-length. Newton proved that white light is composite, and that the rays of different colour, of which it consists, undergo different amounts of refraction, the violet being refracted most and the red least. This angular separation of a ray of white light into its constituent colours is known as dispersion. The following is a brief account of Newton's work.

In the year 1675 Newton made the discovery of the action of the prism upon sunlight in the following manner :—In the shutter of a darkened room he made a small, round hole ; in the path of the sunlight which streamed in through this small hole, he placed a glass prism, and when the base of the prism was turned upwards, he found on a white screen placed opposite the shutter a coloured band changing gradually from red to violet, through orange, yellow, green, blue and indigo. In this way, and by the recomposition of the spectrum, Newton discovered that white light is split up by the prism into its component colours ; he also found that these different colours were not all equally bent by the prism ; so that "the light of the sun consists of rays of different refrangibility". Red light is not so far deviated from its direction as the blue or violet.

This coloured band on the screen was the spectrum of sunlight, but, in consequence of the overlapping of each other by the coloured images of the round hole it was not a pure spectrum, the colours being mixed. In 1802 Dr. Wollaston discovered that if a slit were made in the shutter instead of a round hole, the spectrum of sunlight, instead of being composed of a number of coloured discs, was now a band of pure colours, each colour being free from admixture with the one next to it.

Moreover, he found that this coloured band was not continuous, as Newton described it, but interrupted here and there by fine black lines. In 1814 Fraunhofer, a German optician, discovered these lines independently, and mapped out 576 of them, calling the most prominent A, B, C, D, E, F, G, H, which lines he used as standards for comparison. He also found that the distances of these lines from each other may vary according to the nature of the substance composing the prism; thus their relative distances are not the same in prisms of flint-glass, crown-glass, and bisulphide of carbon, but they always occupy the same position relatively to the colours of the spectrum. Kirchhoff and Ångström have mapped out more than 2000 Fraunhofer lines.

In 1830 Simms, an optician, made an improvement in the construction of the spectroscope by placing a lens in front of the prism so arranged that the slit was in the focus of the lens. This lens turns the light after it has passed through the slit into a parallel beam before it enters the prism. Another lens was also introduced by him which receives the parallel beam emerging from the prism and converts it into a

convergent beam which converges to a focus, forming there an image of the slit which may be magnified at pleasure for each ray. The lens between the prism and the slit is called the collimating lens. Thus the following are the essential parts of a chemical spectroscope :—

1. A slit, the edges of which are two knife-edges of steel or other metal or quartz very truly ground, and exactly parallel to each other, and placed in a direction parallel to the refracting edge of the prism, to admit a pencil of rays.

2. A collimating lens ; a convex lens with the slit at its principal focus, which renders the rays parallel before entering the prism. The tube in which this lens is placed is equal in length to the focal length of the lens, and is called the collimator.

3. A prism of dense glass, in which the parallel rays are refracted and dispersed. It is usual to place the prism so that all the rays are refracted through it with approximately minimum deviation.

A diffraction-grating may be used instead of a prism, but nothing is gained by its use in the case of absorption spectra except for photographing the ultra-violet, for besides its great dispersion, spectra are much less bright than when a prism is used.

4. An observing telescope, constructed like an astronomical refractor of small size, and placed so that the rays shall traverse it after emerging from the prism. It must be focussed as if to view a distant object, because rays of a given refrangibility from a given point of the slit are parallel before entering it.

Such are the essentials of a one-prism chemical spectroscope. In a direct-vision instrument, such as

the Sorby-Browning microspectroscope, the construction is somewhat different, which will be explained when we come to describe that instrument.

When various sources of light are examined with a combination such as has been described, we find that spectra can be classified under three heads.

Spectra may be divided into three groups, (1) emission spectra, (2) absorption spectra, and (3) the solar spectrum. In the first group the character of the spectrum is dependent on the nature of the light emitted from the luminous source ; while in the second, the character of the spectrum is dependent on the kinds of light, which the body absorbs when white light traverses it or is reflected from it.

1. *The Emission Spectra.*—These may be again divided into three classes, (1) continuous spectra, (2) line spectra, and (3) banded spectra.

(1) *Continuous spectra* are emitted by white-hot solid and liquid bodies. They contain light of the whole series of wave-lengths, and appear to the eye as a continuous band of colours arranged side by side, passing gradually through red, orange, yellow, green, blue, indigo, and violet. This type of spectrum is given when we illuminate the slit of the spectroscope with gaslight, candle-light, magnesium-light, limelight, or electric-light.

(2) and (3) *Line and banded spectra.*—Incandescent gases and vapours emit light of certain fixed wave-lengths characteristic of each chemical substance, and consequently yield a spectrum consisting of more or less isolated lines or bands. The bands may be defined from the spectroscopic standpoint as groups of closely approximated lines. The temperature of

the flame of the Bunsen burner is sufficient for vaporizing salts of the alkalies and alkaline earths. When higher temperatures are required, the oxyhydrogen flame, electric arc, or sparks from an induction coil may be used. Textbooks of spectroscopy may be referred to for adequate details.

2. *Absorption Spectra.*—These may be divided into two classes according as the absorbing medium is (1) gaseous or (2) liquid. Gases and vapours selectively absorb those rays which they emit at a higher temperature. The absorption spectra of gases are of great importance in relation to physics and astronomy, but have little bearing on the application of spectroscopy to biology. On the other hand, the absorption spectra of substances in solution will be illustrated by many examples in subsequent chapters of this book.

Colours of Solids and Fluids and their Relation to Absorption Spectra.—The colours of solids and fluids result from their being capable of absorbing certain rays of white light, and of reflecting others; an object is red because it absorbs all rays except the red, which it reflects, and so it appears to be of a red colour. A white object reflects all the rays of white light and so appears to have no colour, while an object is black when it absorbs most or all of the light falling on it. Again, the different colours of transparent solids and fluids result from their capability of absorbing certain rays. A solution of permanganate of potassium transmits the red and blue-violet parts of the spectrum unaltered, while in the yellow and green are black bands; the colour of the fluid itself is reddish-violet, and it transmits the red and violet parts of the spectrum. Again chlorophyll, or the colouring matter of leaves,

gives, when in alkaline solution, a well-marked absorption spectrum, in the middle of the red a black band between B and C, three feeble absorption bands in orange-yellow and green, while the violet and indigo colours are entirely shaded. Sometimes absorption bands appear faint and hazy, at other times sharp and clearly defined. The method of studying absorption spectra will be referred to further on.

3. *The Solar Spectrum.*—This spectrum is composed of the red, orange, yellow, green, blue, indigo (or more probably ultramarine), and violet colours, forming the band which Wollaston got by transmitting the ray of sunlight through the slit and the prism, as before described, so far resembling the continuous spectra of white-hot solids and fluids ; but, in addition these colours are here and there interrupted by the presence of black lines, some of them of exceeding fineness, all these lines being placed at right angles to the length of the spectrum. Some of these lines (which were described by Fraunhofer, as mentioned before, and therefore called Fraunhofer lines) are better marked than the rest, and as they always occupy the same position relatively to the colours of the spectrum, they are taken as marks, to which the position of absorption bands is referred. The first Fraunhofer line, A, is in the red, so also are a, B, C ; D is in the orange-yellow, E and b in the green, F between blue-green and blue, G in the violet-blue, H in the violet, and so on.

Fraunhofer discovered that the bright yellow sodium line occupies the same position in the spectrum as Fraunhofer's line D, and the same experiment is easily performed by any one who possesses a spectro-

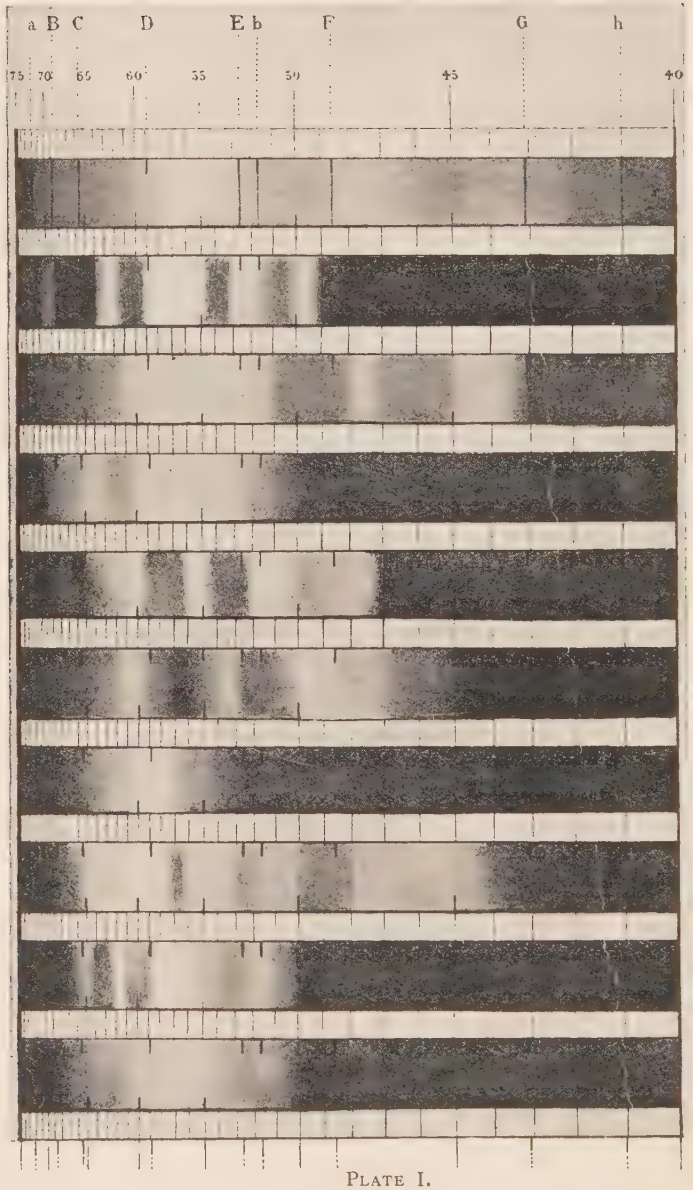

a B C D E b F G h
75 70 65 60 55 50 45 40
Sp. 1
Solar Spectrum.
2
Chlorophyll alcohol 90 per cent. lupinus.
3
Carotin in balsam.
4
Fluorescence of lupinus in alcohol.
5
Turacin in a feather.
6
Spongio-porphyrin in balsam.
7
Fluorescence of Hæmatochrome.
8
Aqs. sol. Hæmatochrome.
9
Hæmatococcus chlorophyll.
10
Fluorescence of do.

PLATE I.

scope, for, by directing sunlight on the slit of the spectroscope, the Fraunhofer lines appear, and then causing the light from a spirit lamp or Bunsen burner, which has in its flame a salt of sodium, to fall upon the right-angled prism covering half of the slit, it will be at once evident that the D line of the solar spectrum is coincident with the bright yellow line of sodium ; and, by a method which in principle is the same as this, it has been shown that most of the lines of the solar spectrum occupy the same position in the spectrum as the bright lines of various chemical elements ; in this way the foundation of solar and stellar chemistry has been laid. But if the Fraunhofer lines are the lines of the incandescent vapours of elements burning in the sun, why are they black? Why is the D line not yellow? In 1859 Kirchhoff discovered that vapours in a comparatively cool state had the power of absorbing the light emitted by the same vapours in an incandescent state ; or putting the law in more accurate language, every gas and every vapour at a given temperature absorbs exactly those kinds of rays which it emits when in the glowing condition, whilst it permits all other kinds of rays to traverse it with undiminished intensity. An experiment proves this law. If the light from the limelight be allowed to fall on the slit of a spectroscope, and then a flame coloured by a salt of sodium be interposed between the light and the slit, a black line appears in the position of the sodium line. Although the causation of these Fraunhofer lines is explained in a great number of books on physics, etc., yet I have thought it right to give a brief epitome of it here, as a great deal of confusion exists in people's minds with regard to this most

important subject. The Fraunhofer lines are of the greatest importance in the study of absorption bands, because the principal ones, as mentioned above, are used as marks to indicate certain parts of the spectrum, with the position of which the position of absorption bands is compared (and from which measurements can be taken), so that we may be able to state that a

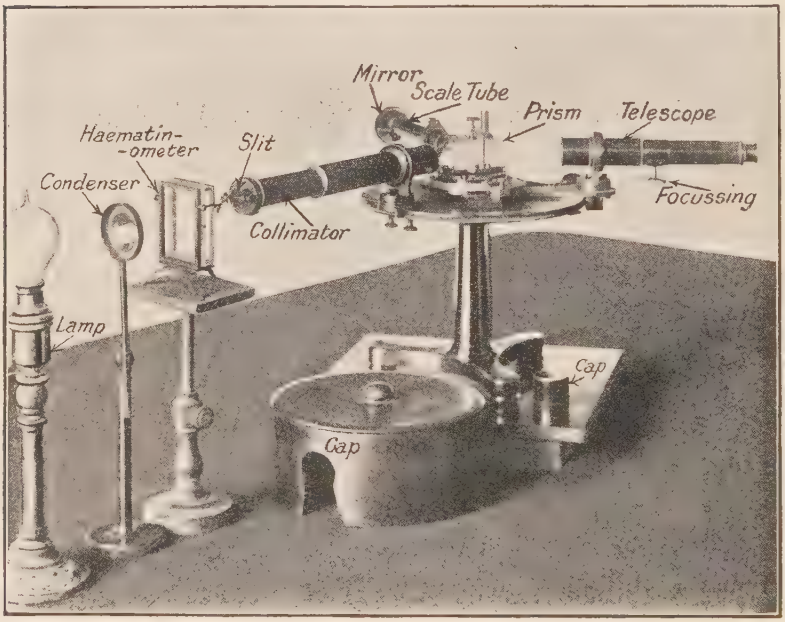


PLATE II.—Dr. MacMunn's spectroscope.

certain absorption band coincides with certain Fraunhofer lines.

The Modern Spectroscope.—We owe to Bunsen and Kirchhoff the instrument which has brought this method of analysis within the reach of the chemist and biologist. The one-prism spectroscope is that which is the most useful for the study of absorption bands,

because in it too great a degree of dispersion is avoided. We want to compress these bands so as to make them as distinct as possible.

I use the instrument here figured. (Plate II, p. 10.) It consists of all the parts mentioned, but in addition it will be seen that the scale-tube is illuminated by a mirror, not by a naked light. The scale may be one

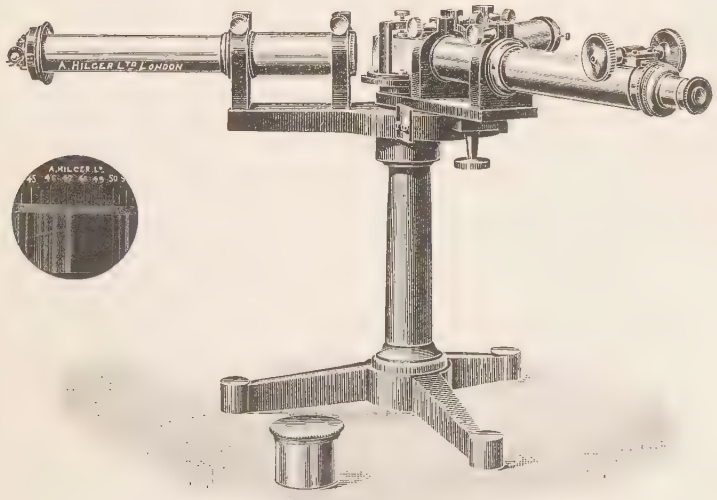


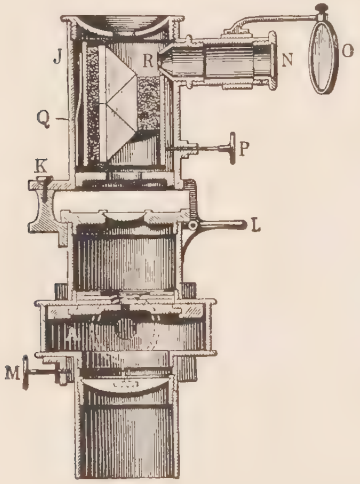
Fig. 1.—Hilger's wave-length scale spectroscope.

of equal parts or it may be a wave-length one such as is made by Hilger (Fig. 1).

In addition to a chemical spectroscope a micro-spectroscope is required for viewing small quantities of material (fluid or solid), and I now invariably use a Zeiss micro-spectroscope which is a direct-vision instrument. It has a compound prism composed of three prisms of crown and flint-glass. Although so small an instrument (Fig. 2), it gives very good dispersion. It is provided with a very finely divided

wave-length scale N which can be set before taking each observation. This is done by setting the number 589 of the scale for the D line of the solar spectrum or for the sodium line. The best power to use with this micro-spectroscope is an inch objective, but of course any other power may be used according to the observer's requirements.

The fluid should be placed in flat-bottomed tubes let into slabs of wood, and the narrower these tubes



O. Mirror for illuminating scale of $\lambda\lambda = N$.
P. Screw for setting scale.
L. Clamp by means of which the upper part of instrument can be turned aside, so as to view the object before observing the spectrum.
Q. Spring for keeping the compound direct-vision prism in place.
M. Clamping screw to fix instrument to the microscope.

FIG. 2.—Zeiss micro-spectroscope.

are, the better, when only small quantities of fluid are available. Sorby's tubes may be used, but they are inconvenient owing to their having to be cemented for each observation.

For comparison, tubes to be clipped by the side-stage clips are convenient.

I may say here that it is absolutely hopeless for anyone to attempt working with a Zeiss micro-spectroscope until he has studied all the parts of

the instrument. It is very complicated, but once its mechanism is mastered, it will be found a perfect instrument.

Application of *diffraction gratings* to biological spectroscopy.—Some disadvantages of the use of gratings for the study of absorption spectra have already been mentioned. On the other hand, gratings possess the advantage of yielding spectra in which the distance between any two lines of the spectrum is directly proportional to their difference of wave-length. They appear to be preferable to the prism-spectroscope for the examination and photographic reproduction of absorption bands in the violet and ultra-violet regions of the spectrum: but the prism-spectroscope seems, on the whole, to give better results in the case of absorption bands in the red end of the spectrum.

Thorpe-replicas (i.e. collodion impressions of original gratings fixed on a plane glass surface) have the advantage over original gratings of being much cheaper. As a rule, the gratings, which have been employed for biological work, have 14,000-15,000 lines ruled to the inch.

The application of gratings to the study of the absorption spectra of pigments of biological importance has been mainly due to the recent work of E. Rost, F. Franz, and Heise ("Beiträge zur Photographie der Blutspectra," 1909). These writers have prepared a complete series of photographs of the absorption spectra of the blood pigments and their derivatives with the aid of a grating. Full details regarding the technique of the method are given in the monograph referred to. Another very useful account of the technique is given in an article by O. Schumm in

Abderhalden's "Handbuch der biochemischen Arbeitsmethoden," Vol. VI, pp. 388-434 (1912).

The following are the wave-lengths of the principal Fraunhofer lines in millionths of a millimetre :—

Line	
A	760
B	686
C	656
D	589
E	526
F	486
G	430
H_1	396
H_2	393

To reduce the readings of an arbitrary scale to wave-lengths we use an interpolation curve. Such a curve is here shown (Fig. 2A). A piece of squared paper about four times the size of that shown has the arbitrary divisions of the scale of the spectroscope (or the angular divisions of the graduated arc) marked off along the top line and the wave-lengths along the right (or left) hand edge. The principal Fraunhofer lines are then dotted down on the map from these data. If possible, other lines such as those of lithium, strontium, barium should also be dotted down and a curve drawn as uniformly as possible through all those points. The points should, if this can be managed, not be less than from 10 to 15.

The spectroscope has now reached great perfection, and I may be allowed to call attention to some very perfect instruments.

Fig. 1 shows an instrument which allows wave-

length measurements to be determined directly; it is made by Hilger. The measurement is here made by

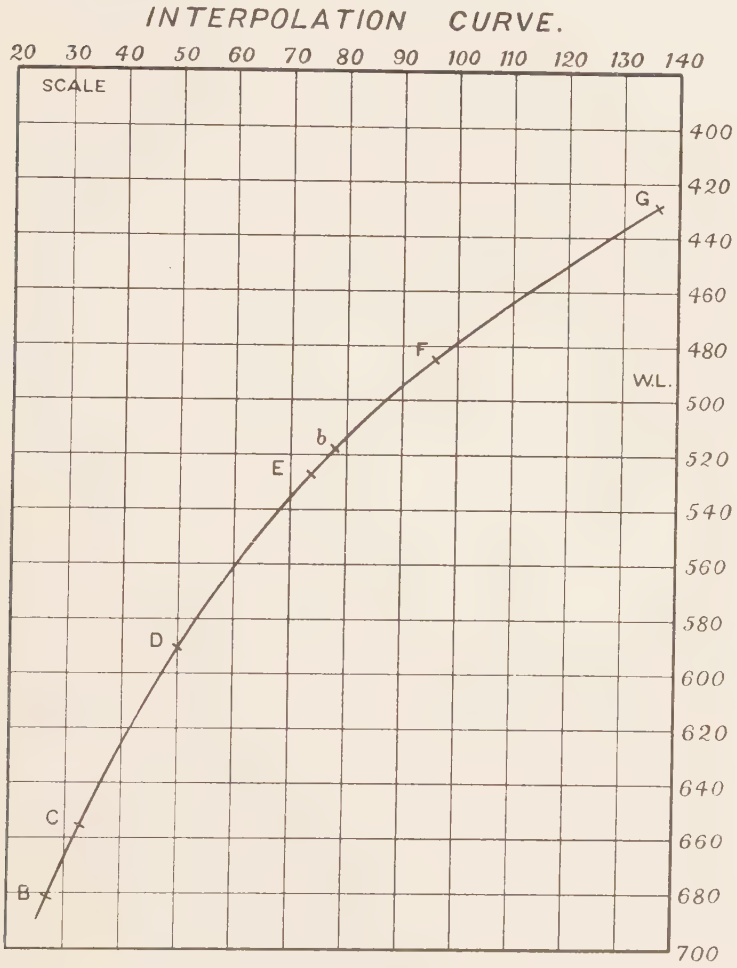


FIG. 2A.

means of a scale-tube. A more elaborate measuring apparatus is given by means of a spiral drum seen in the figure; this latter is a beautiful piece of

mechanism (Fig. 3). Dr. Edridge-Green uses this method of measurement combined with a shutter eye-

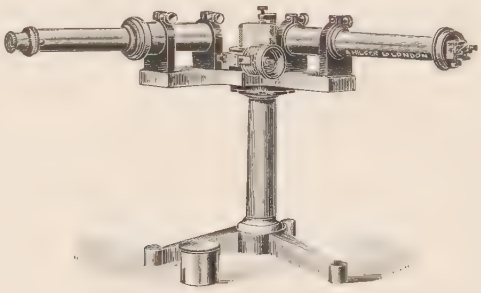


Fig. 3.—Spectroscope with spiral drum.

piece (to be described under spectrophotometry) for testing colour blindness : this is also made by Hilger.

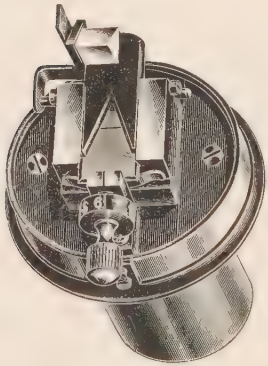


Fig. 4.—Right-angled reflecting-prism for comparing two spectra together.

Another very useful adjunct is that shown here by means of which the depths of the spectrum from above downwards can be increased or diminished. The same figure (4) shows the right-angled reflecting-prism for comparing two spectra together. This is indispensable for chemical spectroscopy. In the same figure is shown the screw for opening and closing the slit. To clean a slit, a metallic instrument must not be used. A slit is known to be dirty by lines running along the length of the spectrum. To clean the jaws, the slit should be opened widely and a bit of wedge-shaped wood cut from a match, or something of that kind should be used.

Another useful adjunct is that here shown. As Fig. 5 shows, the thickness of the layer of liquid can be read off in millimetres. The quartz end plates are for work in the ultra-violet. This is an improvement on the "hæmatoscope" of Herman figured in some of the textbooks of physiology.

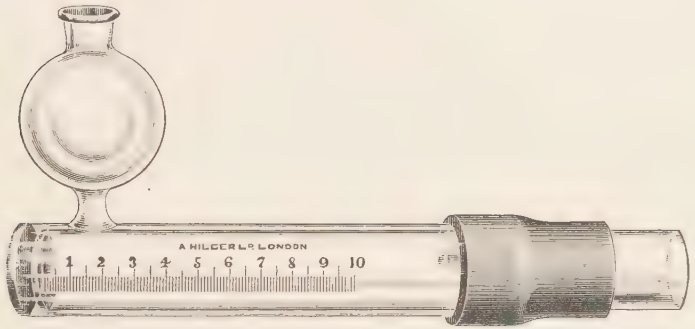


FIG. 5.—Absorption tube.

Spectrographs.—A spectroscope provided with a camera is known as a spectrograph. The accompanying figure shows such an instrument (Fig. 6). The eyepiece of the observing telescope is removed and the camera slipped on. The focussing is done by means of a ground-glass screen and the usual photographer's device. The writer finds that much trouble is saved by photographing the scale in the scale-tube of the microscope simultaneously with the spectrum. (See Fig. 1 accompanying picture of wave-length spectrometer, photographic scale, type p. 11.) The plates of Wratten & Wainwright, Croydon, are very satisfactory. Some are sensitive to the whole range of the spectrum, and are used by Marchlewski and others.

The panchromatic "A" are sensitive from the

ultra-violet to wave-length 7000 Ångström, and are perhaps the most useful for our purpose. The panchromatic "B" are less sensitive and therefore will stand a longer exposure. All must be put into the camera slide and be developed in total darkness. Pamphlets giving full directions are supplied gratis by

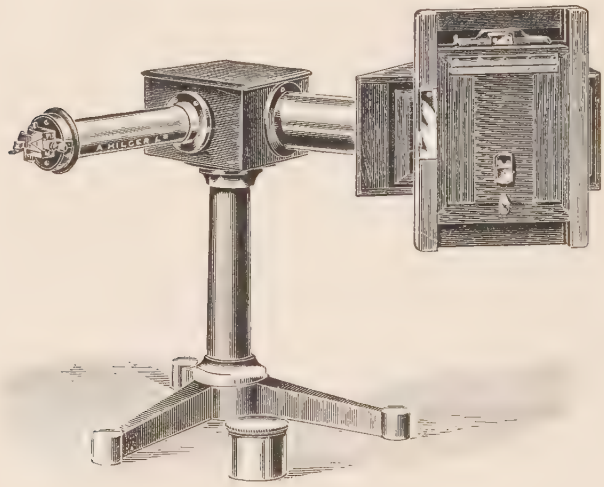


Fig. 6.—Spectrograph.

the makers. It may be remarked here that for photography of the ultra-violet, quartz prisms or diffraction gratings and quartz lenses must be used.

The spectrophotometer and spectrophotometric methods will be described in a subsequent chapter.

CHAPTER II.

GENERAL INTRODUCTION TO THE CHROMATOLOGY OF PLANTS AND ANIMALS — PIGMENT AND STRUCTURE.

The coloration of animals is due either to the presence of colouring matters in, or to a certain structure of, the surface, which latter so affects the light falling upon it as to produce diffraction, or refraction or interference phenomena, or to both combined. In plants the coloration is mainly due to colouring matters. These colouring matters absorb certain elements of the light, and allow the colours not absorbed to emerge and affect the eye of the observer. Thus a red object appears red because all the elements of the light falling upon it are absorbed except the red rays. An object is black when all the rays falling upon it are absorbed, and an object is white when they are not absorbed. Effects of a great variety are brought about by the joint action of what we may call physical and chemical processes, examples of which will be referred to later on.

To restrict our attention to the pigments of animals would give a narrow and prejudiced idea of the rôle which pigments perform. And as our knowledge of the physiological chemistry of plants and animals progresses, we are surprised to find how many substances are common to both kingdoms of nature. The same remark applies to the pigments. By thus

extending our survey to plants as well as animals, we shall, I believe, be in a position to appreciate the immense importance of pigments to the life of the animal as well as the plant.

The visible spectrum of an incandescent light-source is a continuous spectrum passing from red at one end through orange, yellow, green, blue, and violet. If we take a bar of iron and heat it to redness, it emits rays of longest wave-length; these are the results of comparatively slow vibrations. By continuing the heating, the vibrations become more rapid and shorter waves are emitted. If we examine the red-hot iron with the spectroscope, we get only the red rays of long wave-length, and caused by slow vibrations; if we examine the white-hot iron, we get a continuous spectrum produced by more rapid vibrations. But beyond the red and beyond the violet are the infra-red and the ultra-violet parts of the spectrum, the former producing heating effects and the latter chemical effects. The dark heat-rays are caused by vibrations too slow to affect the eye, the ultra-violet, or chemical rays, by vibrations too rapid to affect it, or at least to be perceived by it.

Colour Vision.—Visible colour is the sensation produced in the eye by waves of light striking the retina with a frequency of 757 to 392 billions per second. At the line – in the dark-red of the solar spectrum the wave-length is 760 millionth of a millimetre, at the line H_2 in the pure violet it is 393 millionth of a millimetre. We know that all the colours of the spectrum and each part of these colours are produced by waves of a given length, and when an absorption band blocks out a portion of a certain colour, we say

it extends from wave-length so-and-so to another wave-length. Hence we always express the position and breadth of absorption bands in terms of wave-lengths of light.

We must carefully distinguish, when speaking of animals and plants, between colour or coloration (the arrangement of colour) and colouring matter. "The colour as such," says Poulton, "is not necessarily of any value to an organism." The colour of blood, of fat, of the liver, or kidney, or other internal parts of animals, "has no more meaning than it has in a crystal of sulphate of iron or copper. Such colours are the incidental results of chemical or physical structure, which is valuable to the organism on its own account." Such colours as these are known as "non-significant". At the same time these colours may "form the material out of which natural or sexual selection can create significant colours".

"Significant" colours, on the other hand, are those which are useful to animals in various ways, such as those which are of direct physiological value, e.g. in respiration, or for purposes of mimicry, protective or aggressive resemblance, or as warning colours, or for display in courtship.

Plant colours may also be divided into significant and non-significant. In relation to this subject, Darwin ("Descent of Man") observes: "The tints of the decaying leaves in an American forest are described by every one as gorgeous; yet no one supposes that these tints are of the least advantage to the trees. Bearing in mind how many substances closely analogous to natural organic compounds, and exhibiting the most splendid colours, have been recently formed

by chemists, it must have been a strange fact if substances similarly coloured had not often originated, independently of any useful end thus gained, in the complex laboratory of living organisms".

Pigments and Pigment Precursors.—There is hardly any colouring matter of vegetable or animal origin which does not arise from a colourless mother-substance or chromogen. O. Witt has suggested that the cause of colour in aromatic compounds is due to the presence of a colour-producing group or chromophor in the molecule. "A coloured substance is one which absorbs rays at either end of the spectrum, or selects rays of a definite wave-length from the middle of the spectrum." Every fluorescent substance according to this idea is coloured, "and benzene, benzenoid hydrocarbons, phenols, and other derived compounds which exhibit selective absorption of the violet rays are coloured. The subtlety of the colour, however, is such that the eye cannot perceive it without the aid of photography or a fluorescent screen."[1]

Lord Avebury has shown that ants are apparently capable of perceiving the ultra-violet rays which are quite invisible to our eyes. It is usual to apply the term "coloured" substance to one in which the absorption of rays extends from the visible red to the visible violet end of the spectrum, or roughly from wave-length 700 to 400 millionths of a millimetre. To convert a substance such as benzene with an invisible colour to one with a visible colour, "it is necessary to slacken its rate of vibration, so that the molecule will absorb rays with oscillatio-frequencies (inverse wave-lengths) occurring within the limits of

[1] Hartley. Watt's "Dict. of Chem."

visibility. That which is called a chromogen is an invisibly coloured substance, and that termed a chromophor is an atom or group of atoms capable of so affecting the molecule as to reduce its rate of vibration, so that it absorbs rays within the limits of visibility. Under certain conditions oxygen and nitrogen are chromophors, hence also nitroxyl and hydroxyl, that is to say, they are themselves coloured visibly or invisibly. When two benzene molecules are doubly linked by two nitrogen atoms, as an azobenzene, their mode of vibration is profoundly modified, and a brilliant colour as low down in the scale as the yellow ray is the result. A similar modification takes place when two atoms of oxygen replace two of hydrogen, as in quinone, which is of a golden-yellow colour." These conceptions of Witt may help us to understand how colourless substances are so easily transformed into coloured ones.

Colouring Matters of Plants.—Although such an extensive surface of the earth is coloured green by the presence of leaf-green or chlorophyll, no one has succeeded as yet in obtaining the ingredients of this mixture of colouring matters in a pure condition,[1] and yet those who have worked at such an abundantly present substance are always ready to criticize those who are unable to isolate, and analyse chemically, pigments which are only obtainable in infinitesimal quantities.[2]

In Semper's book, "The Natural Conditions of Existence as they Affect Animal Life," we find the following: "If we except the lowest organisms, the relations between light and the organism seem to be

[1] Cf. Marchlewski. [2] MacMunn, "Ency. Brit."

maintained by two very dissimilar organic structures—by the eye in the animal and by the chlorophyll bodies in plants". If we think for a moment how dependent we are on green plants and on the animals feeding on them, we realize of what immense importance chlorophyll is to us.

Chlorophyll Bodies.—We find it difficult to differentiate between the bodies which contain chlorophyll, known as chlorophyll bodies, and the protoplasm in which they are contained. As Kerner remarks, "the groundwork of the chlorophyll granules differs but little in its structure and composition from the surrounding protoplasm. Like all sharply defined protoplasmic bodies, chlorophyll granules exhibit a pellicle-like thickened outer layer; the inner portion, on the other hand, is formed of a porous mass of reticular or scaffold-like strands, which may be best compared to a bath-sponge. The holes and meshes of this spongy colourless ground-substance contain a green colouring matter, which is dissolved in an oily material, and clothes the continuous small spaces in the form of a parietal layer."

These chlorophyll bodies are carried by the protoplasm in which they occur in the movements of the protoplasm; but I wish to call attention here to the fact that in the plant itself we find chlorophyll held in solution in an oily medium, and, as Kerner remarks, they "appear to be imbedded in protoplasm from their origin until their disappearance" (cf. Strasburger).

It has been ascertained that the different rays of which sunlight is composed, as taught by its spectrum, play a different part in the formation of organic materials in plant cells. Thus the blue and violet rays

of short wave-length help to oxidize the carbohydrates —i.e. they are concerned in destructive metabolism (katabolism); the red, orange, and yellow rays, of greater wave-length, on the other hand, assist the formation of these same carbohydrates,—i.e. they are concerned in constructive metabolism, or are anabolic; while the extreme red rays, of greater wave-length still, possess a heating effect. And in this connexion it is interesting to note that chlorophyll and allied substances possess a red fluorescence, and so are able to transform the violet and blue rays into yellow and red, or even to do more, and transform these rays into heat or ultra-red rays, and so transform light into heat (Kerner and others).

The destructive action of sunlight on bacteria has been lately demonstrated by Marshall Ward and others, and Hardy and D'Arcy have shown that these ultra-violet rays which, as stated above, are katabolic and oxidative, are concerned in the destruction of bacteria. Hence we can see the necessity in both plants and animals of a screen of pigment to protect the underlying protoplasm from the ultra-violet rays. Chlorophyll itself possesses this property to a marked extent, and this is due to the optical property of its yellow constituent or constituents.

Seaweeds.—The action of light in seaweeds is of great interest. These have a "zonal" distribution. Thus seaweeds near high-water mark are green in colour like land vegetation, and lower down between tide-marks there is a belt of olive forms sheltering red plants beneath them. When rocks overhang the bottom, and in small pools, then red forms also occur at this level. At extreme low-water mark, and beyond

it, are found the brown tangles sheltering the red forms again, while at the lowest depth of plant life in the sea, the red forms occur without shelter. Between twenty to fifty fathoms seaweeds become more and more rare, while below that depth their occurrence is exceptional.[1] Now it appears that this regulation of pigment is dependent upon "the nature of the supply of sunlight". At a depth of 200 fathoms according to some, or 700 according to others, the sea is quite dark except for the light given out by phosphorescent animals. It is known, though, that long before this darkness is reached, the quantity of sunlight is reduced in its passage through the water and, what is more important, its quality also. Those rays which are most efficient for assimilation are stopped first, while the hurtful blue and violet rays travel to greater depths.

Now it has been found that while all or mostly all seaweeds of different colour possess chlorophyll, yet in the groups mentioned above, especially in the olive-brown, the blue-green, and the red, the chlorophyll or chlorophylls are reinforced by brown, yellow, or red pigments, which act either by "heightening the susceptibility of the chlorophyll to a diminished supply of the useful rays, or as a protection against a relative excess of the blue rays" (Murray). The latter theory is the one which is generally supposed to be the true one. On this point Kerner says: "In the depths of the sea, however, another optical phenomenon must be taken account of, by which the illumination of chlorophyll granules in the plants growing there becomes in the end a favourable one, and that is the

[1] Murray on "Seaweeds".

fluorescence of erythrophyll, the fluorescence of that red pigment to which the Rhodophyceæ owe their characteristic colour". Now Kerner maintains that the light falling on this pigment or rather mixture is changed by it. It changes the blue rays to yellow, orange, and red, i.e. rays of greater wave-length, so that the chlorophyll granules "finally receive those rays which act as the propelling force in the decomposition of carbonic acid".

I would, however, here call attention to another point which has been overlooked by some, namely, phycoerythrin, or rather the phycoerythrins; the pink and red are of a protein nature and show a very remarkable resemblance to animal respiratory pigments. The aqueous solutions, as Sorby has shown, become decomposed at temperatures varying from 65° to 80° C. Finally Sorby shows that these red algæ contain at least six colouring matters of which five possess "a strong and splendid fluorescence".

The idea that phycoerythrin is respiratory has suggested itself to Hansen, who thinks that it absorbs the dissolved oxygen of the water.[1]

Pigments of Seaweeds.—I may enumerate here the pigments of seaweeds. Different kinds of chlorophylls, the phycoerythrins, phycocyanin, and what has been called phycophaein and phycoxanthin. Phycophaein is soluble in water, phycoxanthin in alcohol, the compound pigment being known as phaeophyll or phycophaein. The last are the pigments of the olive-brown algæ, and in these Hansen (loc. cit.) finds the product of assimilation is not starch but fat. I cannot make out which is Sorby's chloro-

[1] "Biol. Central.," 1894, XXIII, 544, 545.

fucin from recent descriptions, but I hope to make investigations on this point.

Phycocyanin and phycoerythrin have both been obtained in crystals by H. Molisch, who finds that they are of a protein nature.[1]

Other Plant Pigments.—Besides chlorophylls and the colouring matters just mentioned, there are the colouring matters of flowers and fruits and the phlobaphenes or pigments of the bark, those of wood, etc., and of thallophytes. A considerable number are produced by chromogenic bacteria, such as *pyocyanin*, *bacterio-purpurin*, and so on.

The colouring matters of flowers and fruits, according to Hansen, are (1) the soluble "flower-yellow" which only occurs in pure bright-yellow flowers ("anthochlor"); (2) the yellow lipochrome (anthoxanthin); (3) the "flower-red," from which the remaining colouring matters can be obtained in a simple manner; and (4) the chlorophyll green. In yellow, orange, and brown flowers, and sometimes but rarely in blue, the colouring matters are confined to protoplasmic corpuscles. In white, violet, blue, and red, and rarely in yellow, they are dissolved in the cell sap.

The autumnal coloration of leaves is mainly due to xanthophyll or carotin, but in many cases besides, a red pigment (erythrophyll) is present in the leaves, dissolved in the cell sap. It is this erythrophyll to which Kerner, under the name of anthocyanin, attributes the most important function—namely, to protect the "travelling substance" in plants by acting

[1] "Bot. Zeitg.," LIII, 1895, Ite Abt., pp. 131-5, and LII, Ite Abt., 177-89.

as a screen against injurious light-rays, and by transforming light-rays into heat-rays.

There is one class of pigments which is common to both plants and animals, and which undoubtedly plays a most important rôle in both—namely, the class of lipochromes or fat-pigments. They are met with everywhere, from the highest to the lowest plant, and occur in most animals. These will be considered directly. The yellow colouring matter of flowers referred to as anthoxanthin is a lipochrome.

Animal Colouring Matters: Lipochromes.—We must now turn our attention to some important colouring matters of animals, and, as I have just remarked, the lipochromes are common to both plants and animals. In plants some undoubtedly perform the task of screening the protoplasm from the nefarious effects of light-rays of short wave-lengths. They are of various colours, e.g. green-yellow, yellow, orange, or red. Very closely connected with these lipochromes are what Krukenberg calls "lipochromoids," and the latter are closely connected with the "melanoids," which last "lead" to the dark pigments which have long been known as melanins. These pigments, the lipochromoids and melanoids, were found by Krukenberg in invertebrate animals (Gorgonidæ, Mollusca), but there is no doubt that even in man, where we are familiar with the melanin in the skin of the negro, or as fuscin in the choroid, the pigment may, and probably does, owe its origin to another source than hæmoglobin (Delépine).

Carotin or chrysophyll (Schunck) which occurs in crystals is a lipochrome. It and most of the lipochromes are coloured blue by H_2SO_4 when the residue

from their solution is touched in the dry state with a drop of this acid. They also give colour reactions with iodine and with nitric and hydrochloric acids. We must note here, however, the probably close relationship between the lipochromes and the melanins, and in animals both are undoubtedly used to screen the surface from the injurious effects of ultra-violet light, and in the eye to absorb surplus light-rays. In the vertebrate eye lipochromes are found in the retina ; they occupy the outer end of the inner limb of the cones, and here are known as "chromophanes" (Kühne). They occur as highly coloured fat globules, and three colouring matters have been obtained from them : rhodophan, chlorophan, and xanthophan. Kühne called these the stable colouring matters of the retina, but although they are relatively stable when compared with visual purple, which is confined entirely to the outer limbs of the rods, yet like all lipochromes they are ultimately bleached by light. When other lipochromes are thus bleached they seem to change into cholesterin-like substances. (It must be noted that these pigments are bleached when separated from the body.) Now in some of the lower animals, such as starfishes, the beginning of an eye is attended by a deposition of such pigment. In starfishes at least this pigment is a reddish lipochrome, and the eye, e.g. in Astropecten, is merely "a lens supplied with a nerve and lying in a mass of pigment". In Solaster or Asteracanthion the lenses look like brilliant eggs "each in its own scarlet nest". In some species there are as many as two hundred eyes ; but there appears to be no retina, so that they can do little more than distinguish between light and darkness (Avebury).

"Suppose," says Lord Avebury, speaking of such lower forms, "however, some solid and opaque particles of pigment deposited in certain cells of the skin. Their opacity would arrest and absorb the light, thus increasing its effect, while their solidity would enhance the effect of the external stimulus. A further step might be a depression in the skin at this point, which would serve somewhat to protect these differentiated and more sensitive cells, while the deeper this depression the greater would be the protection." But suppose this screen of pigment had the property of transmitting the red, yellow, and a few green rays, and stopping the ultra-violet hurtful rays, would it not be an advantage to these "differentiated and more sensitive cells"? That many invertebrate animals destitute of eyes can distinguish light from darkness and are affected more or less by light has recently been proved by Dr. A. W. Nagel,[1] who experimented on eyeless Lamellibranchiates.

Deep-sea Animals.—It is well known to those who study the fauna of the deep sea that their colours are more evenly distributed than they are in animals living near the surface, and spots, stripes, and markings have a tendency to disappear; most of the fishes are brown or black or other dull colour. On the other hand, cuttlefishes are usually violet, and Gasteropods and Lamellibranchs are transparent and white or pale straw-coloured. But the Crustaceans are remarkable for their red colour, some being scarlet, and these owe their colour to red lipochromes. I need not refer to the colour of other invertebrate animals, but merely

[1] "Biol. Centralbl.," XIV, 1894, pp. 385-90.

give Hickson's summary :[1] "The shades of red occur more frequently than they do in the fauna of any other zone or region, but whether this is in any way connected with the fact that red is the complementary colour to that of the phosphorescent light, in which many of these animals live, is at present difficult to say". We saw that deep-sea seaweeds were red, and had a fluorescence, which Kerner points out is a direct advantage to them in the action of their colouring matters on blue and violet and ultra-violet rays. May it not be possible that the red colouring of deep-sea animals has also something to do with a similar action of their colouring matter on the light? At all events we know that this colouring matter —crustaceorubin (Moseley) —absorbs the violet end of the spectrum and transmits red, orange, and yellow rays, and is a red lipochrome.

Eyes of Animals in the Deep Sea.—Before leaving deep-sea animals we may call attention to their eyes. These "are either very large or very small in the majority of cases. Many animals in caves are quite blind, as a rule, such as the blind crayfish of the mammoth cave in Kentucky, and the blind proteus of the caves of Carniola, and so on. Deep-sea animals are not, as a rule." The conditions in the deep sea are different from those in caves. As Hickson remarks,[2] speaking of the former: "In some regions there is probably a very considerable illumination by phosphorescent light, and it is quite possible that many of the characteristic deep-sea forms may occasionally wander into shallower regions where faint rays of sunlight penetrate, or even that the young

[1] "Fauna of the Deep Sea," p. 66. [2] Loc. cit. p. 68.

stages of some species may be passed at or near the surface of the sea. Taking these points into consideration then, it is not surprising to find that, in the deep seas, there are very few animals, belonging to families usually provided with eyes, that are quite blind." In caves there is no light either phosphorescent or otherwise.

Moseley [1] examined the phosphorescent light emitted by three species of deep-sea Alcyonarians with the spectroscope, and found it to consist of red, yellow, and green rays only. Deep-sea fishes possess two kinds of phosphorescent organs : eye-like organs, occurring in one or two rows down the side of the body "forming a series of bull's-eye lanterns to illuminate the surrounding sea, and various glandular organs that may be situated at the extremity of the barbels or in broad patches behind the eyes or in any other prominent places on the head and shoulders" (Hickson). The deep-sea Crustaceans (some of which possess phosphorescent lanterns), the Alcyonarians, and in a less degree the Echinoderms, and some worms, are also phosphorescent.

Dr. Günther finds among the deep-sea fishes certain peculiar forms, blind and not blind : the latter have exceptionally large eyes, which seem specially fitted to absorb the pale phosphorescent light in large quantities ; while the blind fish, on the other hand, are distinguished by peculiar and sometimes colossal organs on the head which have quite displaced the eyes, and appear to be strongly developed phosphorescent organs. These he believes may be used by their owners, "as torches and other lights are used

[1] "Notes by a Naturalist on the 'Challenger'."

by fishermen, to entice and catch other fish. But just as pirates are attracted by the lights of fishermen and guided to their victims, so the light which these blind fish carry in the two lanterns on their heads to attract their prey may be a beacon to their enemies, and at the same time be of assistance to such fish as can see in their movements generally." However this may be, these blind lantern-fishes have long feelers, beards, and the like, which are probably organs of touch or smell to make up for the want of sight. At all events, in the deep sea, eyes and pigments have to a great extent been preserved, and a close parallel is to be observed between them.

Chlorophyll in Animals.—We may now turn to another subject, namely, the occurrence of Chlorophyll in Animals. Among Protozoans such as Paramoecium, Ophrydium, Stentor, Euglena, etc., some species undoubtedly possess this pigment, and of these some may make it by the agency of their own protoplasm. Among Metazoans, the cœlenterate, Hydra Viridis, and the sponge, Spongilla Fluviatilis, besides probably several salt-water sponges, also contain it. Certain worms, such as Convoluta Schultzii and Chetopterus, perhaps may be said to possess chlorophyll. In all these cases the chlorophyll probably is an organ of assimilation just as it is in plants, but in other cases its presence is accounted for in another way.

Thus I have found a chlorophyll in the so-called livers of many invertebrate animals; this is due to chlorophyll eaten by the animal.

Cantharides Chlorophyll.—Pocklington showed some years ago that the elytræ of Cantharides

beetles contains a chlorophyll, and I confirmed his results. This is a food-product, although structure-colour also plays a part in the coloration of the elytræ. Poulton has recently shown that certain caterpillars contain a chlorophyll or rather a derivative of chlorophyll in their blood, which is utilized in the surface coloration of the larvæ. That the colours of lepidopterous larvæ are largely due to modified plant-pigments derived from food, has more recently been proved by Poulton. He divided the batches of eggs laid by Tryphæna Pronuba into three lots, and fed the larvæ in darkness on green leaves, yellow etiolated leaves, and the white mid-ribs of cabbage. The last lot, where the food contained neither chlorophyll nor etiolin, were entirely unable to form the green or brown ground-colour.[1]

Chlorophyll in the Skin of the Horse.—A more curious thing has recently come under my own observation. In investigating the "dandruff" of the horse, Colonel Smith of Aldershot came upon a yellow pigment. I found that this pigment was modified chlorophyll. Every precaution was taken to exclude surface contamination. Moreover, Colonel Smith found he could increase or decrease this pigment by altering the food of the horse experimentally. The green colour of the sloth is due to a different cause. This green colour is due, as Sorby has shown, to the presence of minute algæ, which die when this animal is kept in captivity. The South American sloth gets its green colour on account of its habit of rubbing itself against the bark of trees (Fountain).

Red Light.—It is a long step from a green leaf to

[1] "Proc. Roy. Soc.," LIV, 1893, pp. 418-30.

the skin of a smallpox patient, and yet there is a most important lesson taught by both, namely, the hurtful influence of ultra-violet light on protoplasm. In the "British Medical Journal" for 7 December, 1895, there is a paper by Dr. Niels. B. Finsen on this subject. He begins by calling attention to the fact that the irritation of the skin produced by sunlight which we formerly attributed to the heat-rays is really due to the chemical (ultra-violet) rays. The electric light also, for instance in the electric welding of metals, may cause a severe inflammation of the skin and mucous membrane of the eye. The inflammation of the skin caused by these rays differs macroscopically from that caused by heat in this most important point, that the injury is followed by pigmentation. Here I may remark that the wandering cells carry up the pigment from the cutis to the epidermis, doubtless to protect the deeper epidermic cells from further harm, the whole process being due to the irritative effect of the chemical rays. Now Finsen finds he can prevent the pitting in the skin of smallpox patients by excluding the chemical rays by filtering the light through red glass or red curtains. This has now been corroborated by subsequent observations. Finsen further calls attention to another fact known to farmers ; when cattle and sheep eat buckwheat and are then exposed to ordinary light they get an eruption on the skin, whereas when they stand in dark sheds no such result follows. Further, it is only light-coloured cattle which are subject to the inflammation, not the pigmented ones, and a white cow tarred on one side did not get the inflammation on that side but only on the other. Here then we have positive proof that pig-

mentation is a direct advantage to an animal, and further that pigmentation can be produced by the action of sunlight on the skin. The pigment acts as a screen to the protoplasm of the animal cell, just as a pigment protects the protoplasm of the vegetable cell.

Negro's Skin.—Discussions innumerable have arisen as to the black of the negro's skin, but there is no doubt that a man with a black skin has a better chance of living under a tropical sun than a less pigmented one, provided both are destitute of clothing, if, as I think is proved beyond doubt, protoplasm protects itself against the chemical rays of the sun by a screen of pigment.

Turning now once more to cave animals, we find that the absence of light has not only in time caused blindness, but a most remarkable absence of pigment from their skins. "They are, without exception, either colourless or nearly white, or, in the case of spiders and insects, much paler than their out-of-door relatives."

Colours of Fishes.—But I now turn to Mr. Cunningham's experiments on flat fishes, which prove beyond doubt that pigmentation is caused by sunlight acting on the skin. In a joint paper by Mr. Cunningham and myself[1] it is shown that by illuminating the under side of a flat fish, which as every one knows, is white as distinguished from the upper pigmented surface, it gets pigmented like the upper surface. I have not space to refer to Cunningham's experiments at length, but they are published now *in extenso*, and afford conclusive proof that pigmentation can be produced by sunlight.

[1] "Phil. Trans.," Vol. 184, 1893, B., pp. 765-812.

A microscopic and chemical examination of fishes' skins and other parts will surprise and delight anyone at the ingenuity which Nature displays in producing the most diverse appearances by means of a few materials. Here we have melanines contained in chromatophores, variously coloured lipochromes and guanin, a body allied to uric acid, in fact a waste-product. Coloration of the most diverse kind is produced by the effect of guanin crystals and pigments. The scattering of the light by the guanin granules produces a dull white, while it is reflected by guanin needles as if from the surface of a mirror. The golden appearance of the iris is produced by such a reflecting layer of guanin needles with a yellow or reddish lipochrome deposited on its surface, while the silvery appearance of the iris is produced by the guanin layer alone. Artists know very well the effect of colouring over a thick layer of white, and in fishes the coloured chromatophores stand out with much greater effect on such a background, which is produced by guanin. Greenish effects are sometimes produced by mixing yellow and black, not by blue and yellow, and all other effects are produced by the combined action upon the light of yellow, red, and other coloured lipochromes, with melanines and guanin. Sometimes the glittering effect produced by the presence of small guanin crystals is enhanced by the presence of large prismatic crystals of phosphate of calcium, as Cunningham and I have shown. The fish displays jewels made from the ashes of its own tissues, and yet they are very effective.

In studying animal pigments I have found that we come upon decomposition products of hæmoglobin

where the animal contains no hæmoglobin. Its source can however be explained as these animals contain what I have called Histohæmatins, which yield such decomposition products. I merely mention this here to show that decomposition products are used for surface coloration. Such a use of a waste-product has just been referred to in the case of fishes, where guanin plays such a prominent part. Some white butterflies, according to Mr. Hopkins, owe their whiteness to the presence of an allied substance, uric acid ; and the yellow ones, of the same family, to a pigment which Hopkins obtained by heating uric acid in sealed tubes.[1]

Turacin.—Another instance of a like kind may be mentioned. The Cape Lory and other plantain-eating birds owe their beautiful red colour to a decomposition product of hæmoglobin, a hæmatin which contains copper instead of iron, as Professor Church has shown, and which he names Turacin. This colouring matter is washed out of the bird's feathers by a shower of rain, leaving the bird practically colourless. Other examples might be brought forward where such decomposition products, or waste-products, are used for coloration. While chlorophyll in plants and hæmoglobin among animals are products of constructive metabolism (anabolism) or synthetic products, and are built up by their respective protoplasms, other pigments used for decorative purposes are mostly examples of destructive metabolism (katabolism) and are waste products. As Geddes and Thomson remark :[2] "In a literal sense animals put on beauty for ashes.

[1] "Phil. Trans.," Vol. 186, B., 661-82.

[2] "Evolution of Sex," p. 23.

. . . In many cases, alike among plants and animals, pigments are expressions of disruptive processes and are of the nature of waste-products."

Chemistry of Pigments.—Of the many pigments which have been described up to the present time, very few have been subjected to elementary chemical analysis, owing to the great difficulties attending their isolation. An extremely small amount of pigment will give rise to a great amount of coloration, and the pigments are generally accompanied by impurities of various kinds which cling to them with great tenacity, so that when one has been thoroughly purified, very little of it remains for ultimate analysis. Most of these substances have been detected by means of the spectroscope, their absorption-bands serving for their recognition, but mere identity of spectrum does not necessarily mean chemical identity, and a few chemical tests have also to be applied before a conclusion can be drawn.[1]

The Chief Chemical Properties of the Lipochromes.—Only a few of the lipochromes found in plants have been fully studied from the chemical standpoint. The most important member of the group is carotin or caroten. It is still uncertain whether the carotins of different origin are identical or not. Willstätter, who has investigated them most thoroughly, found no chemical differences between the carotin isolated from green leaves and that obtainable from the carrot (Daucus Carota). Most of the yellow and red colouring matters of flowers and fruits appear to be chemically closely related to carotin. The pigment obtained from

[1] MacMunn, "Chem. of Colours of Animals," "Ency. Brit.," 11th edit.

carrots is the earliest known and most fully investigated representative of the group. It is a yellowish-red substance readily soluble in most organic solvents such as ether, benzene, carbon bisulphide, as well as in fats and oils. Consequently it is frequently found dissolved in the oils of plants. It is insoluble in water, dilute alkalies, and acids. When pure, it crystallizes in rhombic plates having a melting point of 169° C. It has the formula $C_{40}H_{56}$ and is optically active, having a specific rotatory power of $-30{\cdot}17$ at 15° C. An addition-compound is formed with iodine, having the formula $C_{40}H_{56}I_2$. A characteristic feature is the readiness with which it undergoes oxidation. On prolonged exposure to the air, it becomes decolorized, taking up 21 per cent of oxygen. The colourless product thus formed gives the Salkowski reaction for cholesterine and phytosterines. Its constitution is still unknown; but the similarity of its chemical properties to those of fulven (C_6H_6), the mother substance of a number of coloured hydrocarbons, suggests a structural relationship between the two substances. Thiele has shown that fulven has the following structural formula,

$$\begin{matrix} CH = CH \\ \\ CH = CH \end{matrix} \!\!\diagdown\!\!\diagup C = CH_2.$$

Xanthophyll is another lipochrome produced by the partial oxidation of carotin, and has the formula $C_{40}H_{56}O_2$. It crystallizes in red plates, is readily soluble in alcohol, but, unlike carotin, only slightly soluble in ether. It resembles carotin in being readily oxidized on exposure to the air. It is found along with chlorophyll in the green parts of plants and increases in amount during the autumnal colour changes of the foliage.

The lipochromes of animal origin are yellow-, orange-, or red-coloured substances, which form an ill-defined chemical group. Their chemical structure is still quite unknown. They have, however, certain common properties, which form a basis for the methods used for their isolation and identification. They are insoluble in water, but readily soluble in most organic solvents, e.g. alcohol, ether, chloroform, benzene, oils and fats. Their solutions are yellow or red in colour, and are easily bleached by the action of oxygen and light. Stable compounds are formed with alkalies. These compounds are soluble in ether and chloroform, but not in alcohol. The latter properties have been found to be useful in separating lipochromes from fats, lipoids, and bile pigments. All the lipochromes of animal origin show two, or in some cases three, absorption-bands in the blue and violet region of the spectrum. Concentrated nitric or sulphuric acid converts them into blue, bluish-violet and finally pale yellow-coloured pigments. The lipochromes found in mammals are generally known as luteins, and are the chief source of the yellow colour of fatty tissues. One of them, the lutein of the corpora lutea, has been isolated in the form of crystals.

Chemistry of the Melanins.—The melanins are dark brown or black pigments, which have a very wide distribution in the animal kingdom. Many of them have been shown to be derived from colourless precursors or chromogens. They are insoluble in water, dilute acids, even after prolonged boiling, and organic solvents. They are also unaffected by peptic digestion. On the other hand, they dissolve in dilute alkalies, and in concentrated sulphuric acid. The

foregoing are the chief properties, upon which the methods for their isolation are based. In some cases the pigment granules may be partially separated by mechanical means. All of them contain carbon, hydrogen, nitrogen, and some also contain sulphur and iron. The melanin isolated from horsehair has the following percentage composition, C 60·36, H 5·8, N 11·26, S 3·22. The methods of separation, which have been employed, probably result in chemical alterations in the composition of the isolated pigments.

Some light is thrown on their structure and genesis by the nature of the products resulting from their decomposition. On fusion with caustic potash, indol, scatol, and fatty acids are obtained along with other products (Nencki). On oxidation with potassium bichromate in the presence of glacial acetic acid as a solvent, methyl-dibutyl-acetic acid is formed (Spiegler). This acid is probably a product of the oxidation of a hydrocarbon having the formula $C_{12}H_{24}$. By the action of bromine and hydrobromic acid on a melanotic pigment H. Wolff obtained a hydroaromatic compound named xyliton having the formula $C_{12}H_{18}O$. These results indicate that a complex hydrocarbon together with some indol derivative take part in the formation of melanin. The mode of combination of the sulphur in the molecules of those melanins in which it is found, is still uncertain.

Tyrosine, adrenaline, and tryptophane are all labile substances, which readily undergo oxidation to form pigments. Their oxidation is greatly accelerated in the presence of certain enzymes. In the presence of an enzyme tyrosinase, tyrosine is rapidly converted into a red pigment, which later becomes changed into

a dark brown or black pigment. An enzyme has also been found which has little if any action on tyrosine, but greatly accelerates the oxidation of adrenaline converting it into a dark red pigment (Neuberg). Further, Eppinger has recently shown that the mother substance of a melanin found in a case of melanuria is a pyrrol derivative, which is probably derived from tryptophane by a decomposition of the benzene portion of the indol nucleus, the pyrrol ring being left intact. These facts indicate that tyrosine, tryptophane, and adrenaline all play an important part in the formation of melanins.

CHAPTER III.

CHLOROPHYLL IN PLANTS AND ANIMALS: SYMBIOSIS.

The unusual course is here adopted of taking chlorophyll first instead of hæmoglobin, for this reason, that all life, animal and vegetable, depends upon the presence of chlorophyll. Without chlorophyll the rays of the sun could not act upon the plant. Although some would say that the chlorophyll could act just as well if it had no absorption spectrum (Sachs), it has been proved beyond doubt that the maximum of assimilation corresponds with maximum of light absorption by the green parts of plants in the chlorophyll spectrum. In a later chapter I shall show how the spectrophotometer, in the hands of Engelmann more especially, has thrown a clear light on this and on other points.

To the biologist it does not matter whether pure chlorophyll has been isolated or not. What he is concerned with is the action of light on chlorophyll as it exists in the leaf. The spectrum of chlorophyll as it exists in the leaf is identical with that found in an alcoholic solution of chlorophyll. Marchlewski, who for seven years has been working at the chemistry of chlorophyll, has found that this statement is correct. Of course the position of the absorption-bands is changed by the refractive power of the solvent, this being more marked in one of greater refractive index, such as carbon disulphide.

The extracts of green leaves obtained, for instance, by 90 per cent alcohol is impure (rot-chlorophyll), but consists of at least two pigments, a green and a yellow, or perhaps two greens and two yellows as shown by Stokes, Sorby and others many years ago.

The yellow pigment, xanthophyll, the Chrysophyll of Schunck (Fig. 7) or, as it is generally known,

Fig. 7.—Xanthophyll, Chrysophyll or Carotin.

carotin, was not credited with playing any rôle in plant photo-synthesis, but the discovery of a second maximum in the chlorophyll spectrum as proved by Engelmann has changed our views in this direction.

The spectro-photometric curves given farther on show this most distinctly for land plants, and in the case of seaweeds, the second rise in the absorption curve of the spectrum of the thallus (leaf of land-plants) as it is seen in alcoholic solution in the case of

the red algæ (Rhodophyceæ) is helped or replaced by that of the red pigment known as phycoerythrin. The red algæ occupy the lowest depth of the algal zone, and undoubtedly owe their life to the presence of this red pigment.

Chlorophyll and Hæmoglobin.—That a cow, whose blood contains hæmoglobin, eating grass, must manufacture the former from the latter is patent to every one. How this takes place is mysterious, but much light has been thrown upon it by the researches of Marchlewski, Nencki, Sieber and others. The close relationship between the decomposition products of chlorophyll and of hæmoglobin is now established, and in the opinion of some these are identical.

Chemistry of Chlorophyll.—It would be wearisome to enumerate all the products that have been obtained from chlorophyll, such as chlorophyllan, phyllogin, phycophytin, phylloxanthin, phyllocyanin, chlorophyllanic acid, phytochlorine, phytol, phyllotaonin, etc. The most important derivative, however, is the one discovered by Schunck and Marchlewski, namely phylloporphyrin. It is this which chemically and spectroscopically is the most important, because from it at least the hæmoglobin of vertebrates and of many invertebrates has its origin. It possesses practically the same spectrum and almost the same chemical composition as hæmotoporphyrin which will later be considered fully.

Whether pure chlorophyll has yet been isolated is still disputed by the chemists. The older analyses of Hoppe-Seyler and others have been found incorrect. The most recent analyses of Willstätter and Benz are, according to Stahl, the nearest to the truth, and

furnish the formula $C_{38}H_{42}O_7N_4Mg$. The crystals on incineration leave behind them 5·66 per cent of pure magnesia. Crystalline chlorophyll is readily soluble in absolute alcohol, methyl alcohol, acetone and chloroform, only slightly soluble in ether and benzene when cold, and insoluble in petroleum ether.

A brief exposition of the chemistry of the products of the decomposition of chlorophyll is rendered somewhat difficult by the complexity of the subject and by the fact that many points regarding the composition and structure of its derivatives are still in dispute. Consequently I shall only try to give an outline of the chief chemical results. The following sketch is mainly based upon Willstätter's observations.

Chlorophyll has been isolated in two forms, namely crystalline and amorphous. The amorphous form differs mainly from the crystalline in containing 30 per cent of an unsaturated primary aliphatic alcohol known as phytol, which has the formula $C_{20}H_{40}O$. Crystalline chlorophyll contains no phytol, but in its stead two methoxyl groups to each atom of magnesium. Both forms contain magnesium in complex organic combination not as an ion. The magnesium is readily split off by the action of acids ; but is very resistant to the action of alkalies. The pigments, resulting from the decomposition of chlorophyll, may therefore be divided into two classes : (1) those containing magnesium, which are products of the action of alkalies, and (2) those free from magnesium, resulting from the action of acids.

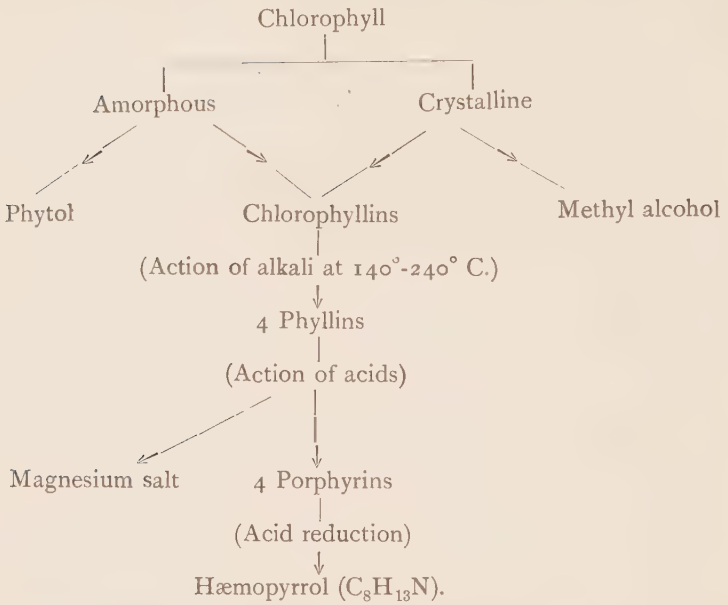
The two chlorophylls are esters. As the result of saponification with alkali in the cold, amorphous chlorophyll yields phytol and the alkaline salts of pigments

having an acid character, which are known as chlorophyllins. Crystalline chlorophyll yields the same pigments, but methyl alcohol instead of phytol. When the chlorophyllins are heated in a closed vessel with a concentrated solution of caustic potash in methyl alcohol the temperature being gradually raised from 140-240°C., a series of blue and red pigments is formed. All these pigments contain magnesium in organic combination. Willstätter and Fritzsche have isolated from this mixture four allied pigments, to which they have given the names glaukophyllin, rhodophyllin ($C_{33}H_{34}O_4N_4M_g$), pyrrophyllin and phyllophyllin. When these four phyllins are acted on by an acid, the magnesium is split off, and a series of porphyrins are formed, named glaukoporphyrin, allo- or rhodoporphyrin, pyrroporphyrin, and phylloporphyrin. Schunck and Marchlewski had previously established the formula $C_{32}H_{34}N_4O_2$ as that of phylloporphyrin. When phylloporphyrin is reduced by means of hydriodic acid in the presence of phosphonium iodide, it yields hæmopyrrol (probably a methyl-propyl-pyrrol). It will be seen later that hæmopyrrol, as its name indicates, is also a derivative of blood pigment.

The tabular scheme on page 50 gives a general view of the relations of these derivatives.

Those who wish to get a good résumé of this difficult subject will get it in a little brochure by Stahl entitled "Zur Biologie des Chlorophylls".

The Action of Light on the Living Leaf and the first Products of Photosynthesis.—The spectrum of chlorophyll consists of six bands and an end absorption which was reckoned by Krauss as the seventh band. The first three or four bands belong to the



green constituent, the others to the yellow. This six-banded chlorophyll then absorbs the rays of the sun in order to work up the carbon dioxide of the air into starch. This process of assimilation, it is needless to say, is quite a different thing from the respiration of plants. Not all parts of plants nor all plants can take the carbon dioxide from the air; only those which contain chlorophyll can do so. "The chlorophyll bodies in themselves are the laboratories in which this chemical process, so important for the whole living world, is carried on. From these laboratories is derived the whole of the carbon which composes the organic substance of all living things, plants as well as animals" (Strasburger). As is well known, this process cannot take place in the dark. In the light, sugars belonging to the hexoses are formed

first, for instance dextrose $C_6H_{12}O_6$. The process of assimilation may be represented thus—

$$6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2.$$

Water is seen to be a necessity for assimilation. The bye-product of the assimilatory process is oxygen; and the volume of oxygen given off is equal to or may exceed that of the carbon dioxide absorbed.

Thus we have plants dependent on the sun for the formation of chlorophyll, and this beneficent pigment not only feeds animals, but enables them to breathe and so to live.

Symbiosis.—"Some plants forego all attempts to develop an adequate chlorophyll apparatus, and by doing so become unable to provide themselves with nourishment from the inorganic matter about them" (Strasburger). The word symbiosis is derived from *σὺν* together, and *βίος* life. But symbiosis is not limited to plants, and just as in plants so in animals it leads to laziness on the part of the animal, which is too ready to avail itself of the association.

That a living plant should flourish within the body of an animal, and that both plant and animal should profit by the partnership, is a strange fact in Nature. But this is now proved to be the case, and the occurrence of symbiosis, as the living together of plant and animal is called, has been proved to occur in many of the lower animals. As symbiosis is the cause of the appearance of a kind of chlorophyll in some animals, I shall now endeavour to give a short account of the subject; and to most people who take an interest in biology the matter is one of extraordinary fascination.

Since Schultze discovered chlorophyll in Planarians, its presence in animals has been put beyond doubt,

as already stated, by the researches of Sir Ray Lankester and Sorby: but among the chlorophyllogenous animals enumerated by Lankester in "Sachs's Botany" (2nd Eng. ed.), Bonellia, Chætopterus and

Fig. 8.—Symbiotic algæ in tentacles of anemone.

Idotea viridis have been found to contain pigments differing from, but probably allied to, chlorophyll. Anthea Cereus, which Lankester and Sorby found to contain chlorophyll, owes its chlorophyll to symbiotic

algæ. The late Professor Krukenberg thought the pigment extractable from the tentacles of the Anthea Cereus was what he called a "hepatochrome," but as the Anthea possesses no liver the term falls to the ground.

The accompanying figure (Fig. 8) shows the presence of these symbiotic algæ within the ten-

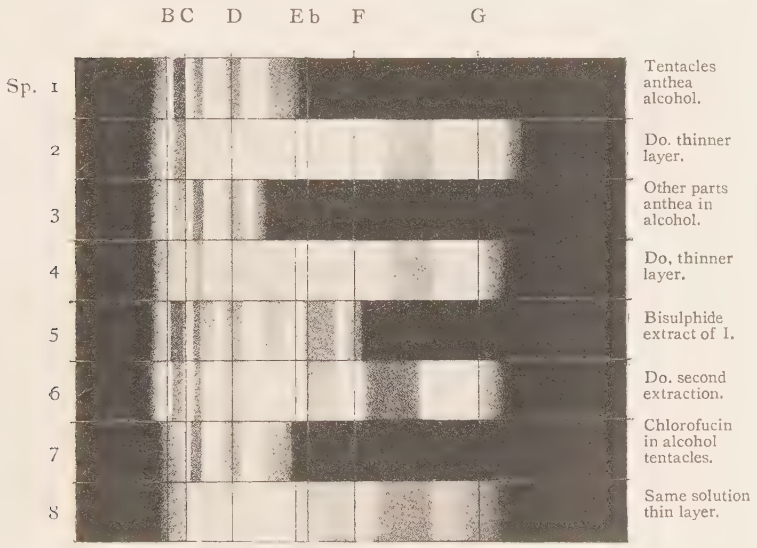


Fig. 9.—Spectra of solutions.

tacles of the anemone, and the algæ were found to possess a cellulose wall and to contain starch.

The spectra of the various solutions are here shown (Fig. 9). From these it is noticeable that more than one colouring-matter is present and in addition to chlorophyll "chlorofucin" is present (Sp. 7).

The radiating cracks in the contents of the tentacle are noticeable, and apart from the proofs show that the algæ are not part of the anatomy of the anemone.

The occurrence of chlorophyll in animals is not only

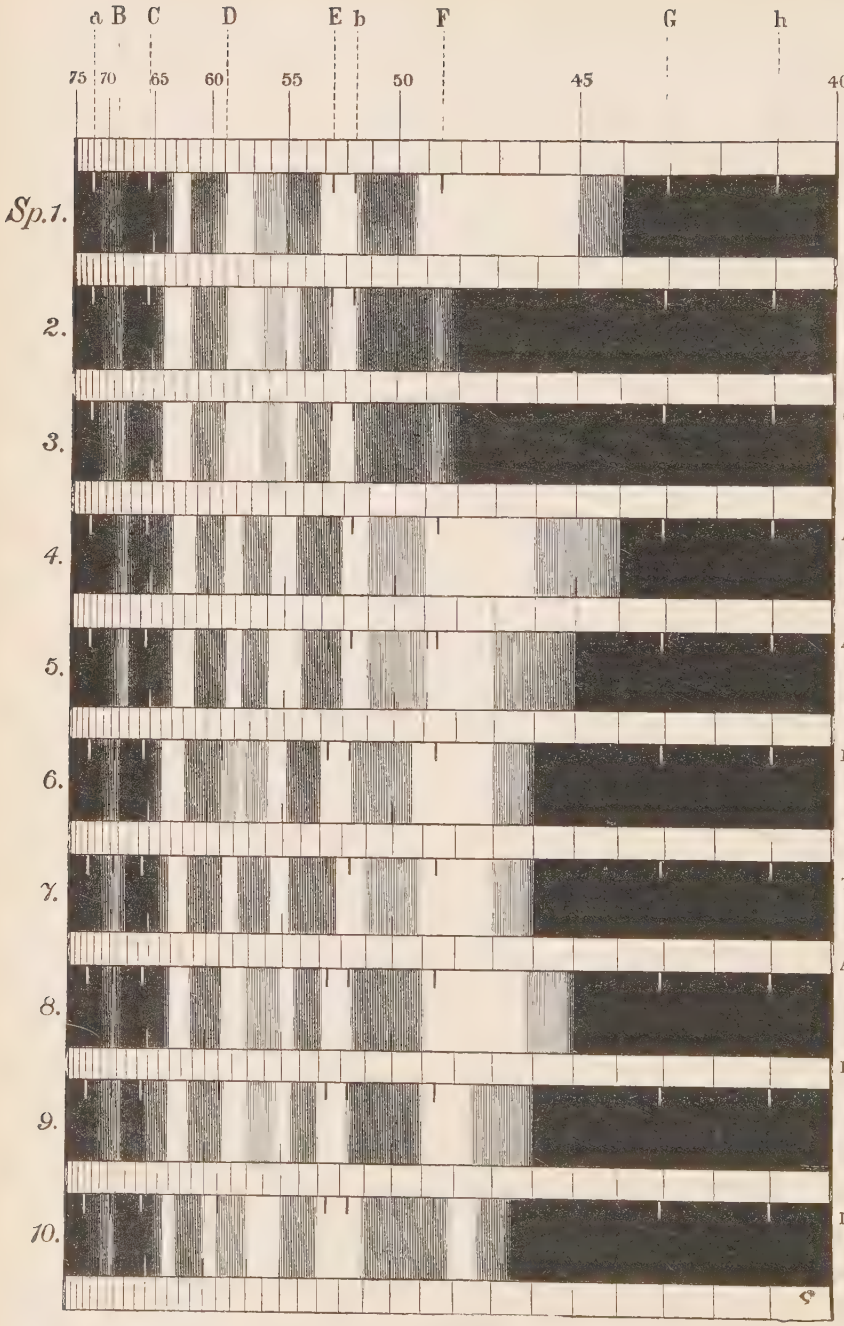
due to these symbiotic algæ, but to chorophyll eaten by the animal. The term "enterochlorophyll" was introduced by me, and since then the term has undergone alteration. One authority calls it "enterochrome" and another "hepatochromate". Since, however, as already stated, there is no liver in an invertebrate the old name had better remain. The whole subject of the occurrence of chlorophyll in animals is discussed by Dr. Otto von Fürth in his encyclopædic book, which is a valuable mine for the biologist.

The researches of Gamble and Keeble on Convoluta Roscoffensis are among the most recent, and these authors have arrived at the conclusion that (1) Convoluta exhibits four stages of nutrition passing from the typically animal to the completely parasitic. (2) The infecting alga shows specialization in the direction of saprophytism. (3) This habit enables the green cells to utilize the products of the animal's nitrogenous metabolism, and so to develop rapidly within the body, where they serve as an excretory system to the animal. (4) The habit of the infecting alga in its free state of frequenting the egg-capsules and also the surface slime of the body of Convoluta, is to be ascribed to its nitrogenous requirements: this habit, developed originally with no reference to Convoluta, being shared with various Chlamydomonadæ, e.g. Carteria Subcordiformis, was nevertheless an essential preliminary to the association of animal and green cell. (5) The association entails ultimately the death of the green cells and of Convoluta; but whereas the former dies without issue, the latter first produces one or more batches of eggs. (6) The consequences of the association are: to the green cell,

hypertrophy, nuclear degeneration, premature senescence; to the animal, suppression of excretory system, cessation of feeding, resignation of power of existence apart from the green cells, i.e. obligate parasitism, adaptations facilitating the photosynthetic activities of the green cell such as marked positive phototropism identical with that displayed by the infecting alga in its free state. Finally, the green cells serve as an excretory system to the animal. The animal, in fact, is too well off, and it degenerates accordingly. It becomes a parasite on its alga and both lose by the association.

Enterochlorophyll.—This has already been referred to and need not delay us long. It is like Chætopterin, an immediate food product. I have proved this by spectrophotometry.

The occurrence of chlorophyll in the skin of a horse has also been referred to, and also the occurrence of chlorophyll in the skin of the South American sloth. Here it is due to an alga concerning which that nature-trained naturalist Paul Fountain observes: "When newly captured, the fur of sloths is often covered with a greenish powder which consists of minute algæ or fungi clinging to the fur. It has been supposed that this fungus grows naturally on the animal's hair; I, however, think it only lodges there in consequence of the animal rubbing itself against the trunks of the trees. It quickly disappears when the animal is kept a prisoner." "Sloths are sometimes green, owing to the presence in their hair of an alga, perhaps the food of the larvæ of small moths which breed in the fur of these remarkable animals" (Poulton). The accompanying plate shows the spectra of Chætopterin, of enterochlorophyll, etc.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75 70 65 60 55 50 45 40
Sp.1.
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10.
Chætopterin in glycerine.
Chætopterin in absolute alcohol.
Contents of gut of Chætopterus in absolute alcohol.
Alcohol solution of Chætopterin acidulated with HCl.
Alcohol solution of the contents of gut of Chætopterus with HCl.
Entero - chlorophyll of Aplysia's gastric gland in absolute alcohol.
The same solution acidulated with HCl.
An ether solution of the precipitate produced by HCl in excess: similar solution.
Precipitate by HCl, isolated and dissolved in hot NaHO sol. Excess of acetic acid added, agitated with ether, and let stand.
Long action of HCl on an alcohol solution of same entero-chlorophyll.

PLATE III.

CHAPTER IV.

HÆMOGLOBIN: ITS COMPONENTS AND DERIVATIVES.

The pigmented protein hæmoglobin, which, as the respiratory pigment, is indispensable to all the vertebrate animals except two, as Sir Ray Lankester has shown, also occurs in the invertebrate.

The following table (p. 70) from von Fürth shows the distribution of this pigment in the invertebrates so far as observed up to 1903.

Considerable caution is needed in determining its presence, an attempt should be made to detect the presence of hæmin crystals and of alkaline, reduced hæmatin and acid hæmatin and of hæmatoporphyrin before finally concluding its presence. The spectra mentioned in this and in following chapters are given in Chart I.

Besides hæmoglobin there are other respiratory proteins such as hæmocyanin, perhaps the writer's echinochrome, Lankester's chlorocruorin, hæmerythrin, and colourless proteins such as Griffith's achroglobine.

The following is, according to Preyer, the empirical composition of oxyhæmoglobin expressed in percentages :—

C, 54·0; H, 7·25; N, 16·25;
Fe, 0·42; S, 0·63; O, 21·45.

Preyer assigned the empirical formula

$$C_{600}H_{960}N_{154}FeS_3O_{170}$$

Preyer gives the molecular weight as 13,232 (Gamgee).

The precise centesimal composition of hæmoglobin is as yet unknown. According to Jaquet the hæmoglobin of the dog is :—

$$C_{758}H_{203}N_{195}S_3FeO_{215}$$

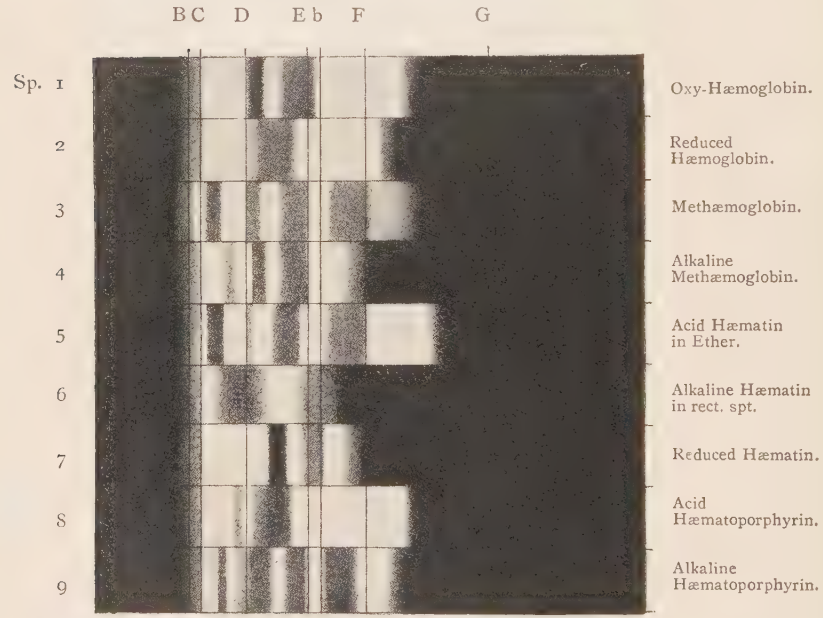


CHART I.—Hæmoglobin and its derivatives.

Absorption of Light by Oxyhæmoglobin.—The visible spectrum of oxyhæmoglobin is known to all readers of modern text-books of physiology.

The invisible spectrum can only be seen by photographing the spectrum produced by the passage of white light through a quartz prism, an absorption vessel containing a dilute solution of oxyhæmoglobin

being placed between the light and the slit.[1] Soret's band is in this part of the spectrum and has been photographed from a dilute solution of oxyhæmoglobin. I have used a fluorescent eye-piece, but found it unsatisfactory.

Arterial defibrinated blood should be diluted with from 400 to 600 volumes of distilled water to which a 0·1 per cent of a solution of sodium hydrate is added (Gamgee).

"No colouring matter yet investigated exhibits the intense absorption bands between G and H which is characteristic of hæmoglobin and its compounds," for instance hæmatin, hæmatoporphyrin and the cuprohæmatin compound known as Turacin.

Reduced Hæmoglobin.—The simplest method of reducing hæmoglobin is got by adding freshly prepared ammonium sulphide. Stokes's fluid is sometimes used, but it has to be prepared for each observation. Hydrazine hydrate is also frequently used as a reducing agent.

Absorption of Light by Reduced Hæmoglobin.—Solutions of reduced hæmoglobin are dichroic. In thick layers, or in concentrated solutions, reduced hæmoglobin is of a dark cherry-red colour while in very dilute solution it is of a green tint.

Compounds of Hæmoglobin with Gases. (1) *Carbonic Oxide Hæmoglobin or Carboxy-hæmoglobin.*—Blood treated with carbonic oxide, if the gas is sufficient in amount, assumes a cherry-red colour and cannot be reduced by Stokes's fluid, ammonium sulphide, etc. It does not, like arterial (oxygenated) blood, change in a few hours to a venous tint but remains red for a

[1] A diffraction grating may replace the quartz prism.

long time. The gas (CO) has entered into a more stable union with the hæmoglobin and displaced its oxygen. The vapour of burning charcoal owes its deadly effect to CO, and coal-gas has been responsible for many deaths owing to its large percentage of this gas. The addition of a concentrated solution of caustic soda to blood saturated with CO (two parts NaHO to one of blood) leads to a scarlet colour and gives a "cinnabar-red" precipitate. Normal blood thus treated is changed into a black shining mass.

Nitric Oxide Hæmoglobin.—Nitric oxide hæmoglobin (NO-hæmoglobin) is like the former, a stable kind of hæmoglobin as NO has a great affinity for oxygen. Blood saturated with NO has a "florid" colour. Whereas in the case of CO-hæmoglobin, the two absorption bands are not in the same place as those of oxyhæmoglobin, Gamgee has found that those of NO-hæmoglobin occupy the same place as those of oxyhæmoglobin. Reducing agents produce no effect as in the case of CO-hæmoglobin, but both pigments give the ultra-violet band (Soret's band).

Like CO-hæmoglobin, NO-hæmoglobin can be crystallized, and yields crystals of the same form.

Supposed Compounds of Hæmoglobin with Other Gases.—Hydrocyanic acid and cyanogen have been supposed to enter into a combination with hæmoglobin by some, but Gamgee doubts it. The same remark applies to acetylene and carbon dioxide.

Derivation of Hæmoglobin and Products of its Decomposition and those of Reduced Hæmoglobin.—When oxyhæmoglobin (O_2Hb) is decomposed in the presence of air the coloured product of decomposition is known as hæmatin. Other uncoloured products arise

at the same time, e.g. globin, the uncoloured part of the oxyhæmoglobin molecule.

The coloured products of the decomposition of hæmoglobin, when oxygen is completely excluded, differ according to the reaction of the solution. When reduced hæmoglobin is decomposed by heating with alkalies the pigment formed is hæmochromogen (Stokes's reduced hæmatin). On the other hand, when even a dilute mineral acid is used for the decomposition, the reduced hæmatin first formed is rapidly deprived of its iron, and converted into hæmatoporphyrine (Hoppe-Seyler and Laidlaw).

Methæmoglobin.—This is a body intermediate between oxyhæmoglobin and hæmatin, and has been a subject of dispute for a long time. Its spectrum is very like that of acid hæmatin, but if reducing agents, such as those mentioned above, be added to a solution of methæmoglobin, at first a spectrum taken for HbO_2 transiently appears and then that of reduced hæmoglobin. Methæmoglobin forms on the edges of healing wounds, on a filter-paper through which blood solution has been filtered, in cases of hæmorrhage from any part of the urinary tract, after extensive burns, and in the urine. It is found also in cases of hæmorrhage into the stomach, etc., in fact in any case where blood comes in contact with a feeble acid. Although so like hæmatin in its spectrum, it is yet a distinct body. The oxygen cannot be removed from it *in vacuo* by the air pump. Gamgee first showed that it is produced by the action of nitrites.

Methæmoglobin is usually prepared by the action of potassium or sodium ferricyanide on oxyhæmoglobin. Haldane gives the following equation to represent the reaction :—

$$\underset{\text{(Oxyhæmoglobin)}}{HbO_2} + 4Na_3(CN)_6Fe + 4NaHCO_3 =$$

$$\underset{\text{(Methæmoglobin)}}{HbO_2} + \underset{\text{(Sodium ferrocyanide)}}{4Na_4(CN)_6Fe} + 2H_2O + 4CO_2 + O_2.$$

If the carbon dioxide, given off in the course of the reaction, be fixed by the addition of a very dilute aqueous solution of ammonia (1 in 500), the amount of oxygen evolved as a gas is found to be the same as that originally present in combination with hæmoglobin as oxyhæmoglobin.

Besides the acid spectrum it has an alkaline one even more characteristic than the former which shows a band in the ultra-violet (Gamgee).

Hæmatin.—If we add strong acetic acid to blood and shake with ether, etc., a solution of a brownish red colour is obtained which shows the spectrum of acid hæmatin. If blood is treated with alcohol and a caustic alkali and examined with the spectroscope, the spectrum of alkaline hæmatin is obtained, and on treating this with reducing agents, such as those mentioned before, the spectrum of reduced hæmatin or hæmochromogen can be got.

This spectrum of reduced hæmatin or hæmochromogen is positive proof of the presence of blood : no other colouring matter will give it. On shaking with air, the distinctive bands disappear, and again reappear on standing owing to the renewed action of the reducing agent.

Hæmochromogen or reduced hæmatin resembles hæmoglobin in forming compounds with carbon monoxide and with nitric oxide. The relation between the amount of gas, entering into combination, and the amount of iron present in the pigments appears to have the same value for all three pigments. The

compounds of these gases with hæmochromogen resemble the corresponding compounds with hæmoglobin in having similar absorption spectra, but differ from them in being very unstable, rapidly decomposing into oxy-hæmatin and free carbon monoxide or nitric oxide on exposure to the air.

Contradictions as to the chemical formula of hæmatin have been numerous. According to Cloettà and Rosenfeld the formula of hæmatin is :—

$$C_{30}H_{34}N_3FeO_3$$

i.e. one atom of iron for three of nitrogen.

On heating dry hæmatin it yields pyrrol : this is another proof of the relationship of animal hæmoglobin to plant chlorophyll (cf. Marchlewski).

By dissolving hæmatin in concentrated sulphuric acid it is converted into hæmatoporphyrin and loses its iron.

Isolated hæmatin is amorphous, and dark brown or bluish-black. It can be heated to 180° C. without decomposition ; on incineration it leaves a residue of oxide of iron. It is insoluble in water, dilute acids, alcohol, ether, and chloroform ; but slightly soluble in warm acetic acid, also in acidified alcohol or ether. It dissolves in alkalies even when they are dilute. The alkaline solutions are dichroic, in thick layers red, in thin greenish.

Hæmin.—This has just been mentioned. It has been known as Teichmann's crystals, and is of great importance not only in the detection of blood in medical jurisprudence, but also in proving the presence of hæmoglobin among the lower animals. It can easily be obtained in the case, e.g., of Arenicola,

Lumbricus, etc. No one seems to know its exact composition. Nencki gives the formula

$$C_{34}H_{33}N_4FeO_4Cl.$$

This has been called "acet-hæmin". Kuster's new formula adopted by the most recent authorities is $C_{34}H_{32}O_4N_4FeCl$.

Hæmatoporphyrin.—This has the formula according to Zaleski of $C_{16}H_{19}N_2O_3$ or $C_{34}H_{38}N_4O_6$. It may easily be obtained by acting on the blood with strong sulphuric acid, filtering through asbestos fibres, diluting the filtrate with water and adding ammonia to alkalinity. A brown precipitate falls which is collected by filtration, dried and then dissolved in various solvents. A purer form of this pigment may be obtained by the action on hæmin of a saturated solution of hydrobromic acid in glacial acetic acid (Nencki).

Hæmatoporphyrin occurs in many of the lower animals (invertebrates) in which no hæmoglobin occurs as I have shown, e.g. in Solecurtus, in the dorsal streak of the Lumbricus, in the integument of slugs, in starfishes, etc. It appears to be identical with Moseley's polyperythrin. Hæmatoporphyrin occurs in small quantity in human urine according to Garrod. It was first detected there by me in various diseases, and Salkowski found it in sulphonal poisoning (cf. "Zoja. Arch. Ital. di Clin. Med.," 1893).

Urine containing hæmatoporphyrin may be almost colourless. Garrod's method of getting it from urine is the most reliable. Under the name "Urospectrin," Saillet has described the same pigment.

Hæmatoidin.—This crystallizes in orange-coloured rhombic plates (see Fig. 10). It is identical with bilirubin the principal colouring matter of human bile.

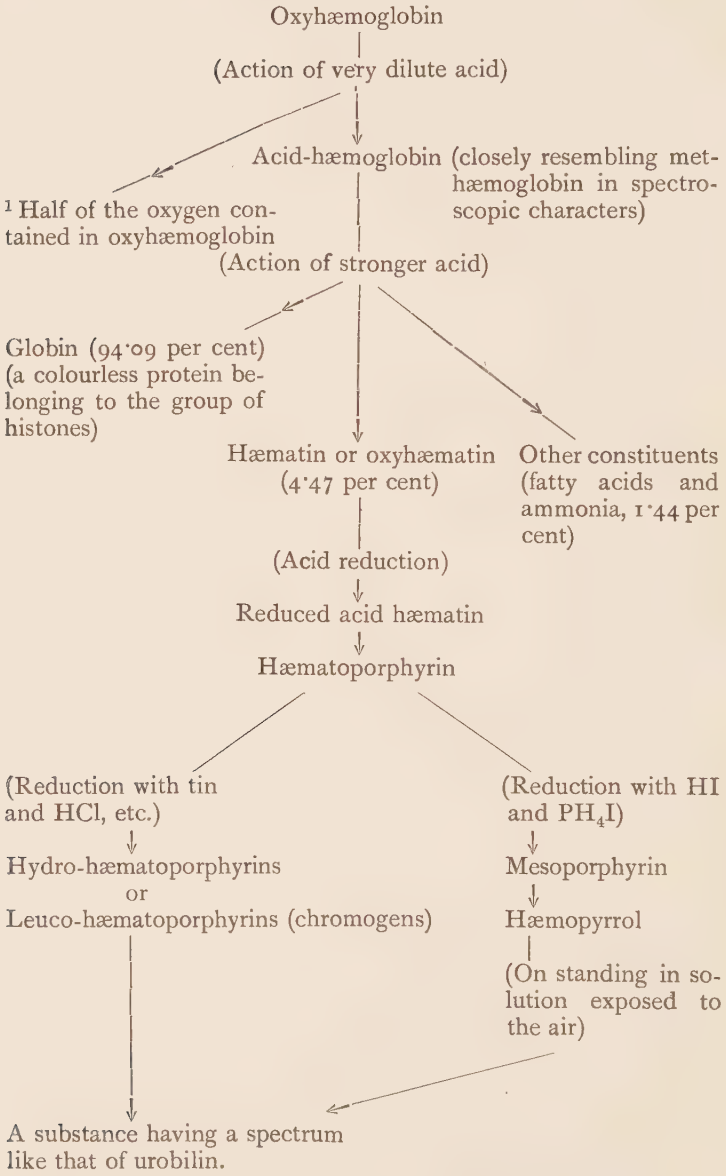
At one time it was supposed to be identical with a "lutein" occurring in the mammalian ovum, in the corpus luteum, but this is not the case. Hæmatoidin gives a colour play with strong nitric acid which led to the mistake (Gmelin's reaction). It has been found, however, in the placenta of the pig in the amorphous state by Mr. Jenkinson of Oxford, and it is said that biliverdin has been found in the placenta of the bitch.

Fig. 10.—Hæmatoidin.

This has been disputed. It occurs in birds' egg-shells, but wherever found it is derived from hæmoglobin.

For the absorption-relation (A) hæmatoidin = bilirubin, see the chapter on Spectrophotometry.

A Synopsis of the Chemistry of the Blood Pigments.—A general view of the products of the decomposition of hæmoglobin is probably best acquired by studying a tabular scheme. The following scheme shows the products of the decomposition of oxyhæmoglobin by acids and acid-reducing agents.


Oxyhæmoglobin
(Action of very dilute acid)
Acid-hæmoglobin (closely resembling methæmoglobin in spectroscopic characters)
[1] Half of the oxygen contained in oxyhæmoglobin
(Action of stronger acid)
Globin (94·09 per cent) (a colourless protein belonging to the group of histones)
Hæmatin or oxyhæmatin (4·47 per cent)
Other constituents (fatty acids and ammonia, 1·44 per cent)
(Acid reduction)
Reduced acid hæmatin
Hæmatoporphyrin
(Reduction with tin and HCl, etc.)
Hydro-hæmatoporphyrins
or
Leuco-hæmatoporphyrins (chromogens)
(Reduction with HI and PH_4I)
Mesoporphyrin
Hæmopyrrol
(On standing in solution exposed to the air)
A substance having a spectrum like that of urobilin.

[1] Possibly the oxygen is not given off until the second stage, in which acid hæmatin is formed.

The most important facts regarding the properties of hæmatin have already been mentioned. Zaleski gives the following equation as representing the conversion of hæmin into hæmatoporphyrin by the action of hydrobromic acid :—

$$C_{34}H_{32}N_4FeClO_4 + 2H_2O + 2HBr = C_{34}H_{38}N_4O_6 + FeClBr_2$$

The empirical formula of mesoporphyrin given by Zaleski is $C_{34}H_{38}N_4O_4$. The molecule of mesoporphyrin will be seen to contain two atoms of oxygen less than hæmatoporphyrin. The empirical formula for phylloporphyrin, given in the section on the chemistry of chlorophyll, shows that less oxygen is present in that pigment than in mesoporphyrin. Close chemical relationships between the three substances are indicated by the similarity of their empirical formulæ and of their absorption spectra. Further, all three pigments yield hæmopyrrol on more complete reduction. The exact composition and nature of hæmopyrrol is still uncertain. It appears to contain pyrroline as well as pyrrol derivatives (Küster). The formula previously given in this book was that proposed by Nencki, who first investigated this substance.

The products of the reduction of hæmatoporphyrin differ according to the nature of the acid-reducing agents used. The right side of the scheme gives the products of the reducing action of hydriodic acid and phosphonium iodide on hæmin or hæmatoporphyrin dissolved in glacial acetic acid ; while the products on the left side are those resulting from the reducing action of various metals, e.g. tin or zinc dust on hæmin or hæmatoporphyrin dissolved in alcohol acidified with hydrochloric acid, or in glacial acetic acid. The substances which are formed under the latter condi-

tions are compounds resulting from the addition of several hydrogen atoms to the molecule of hæmatoporphyrin. Nencki and Zaleski have analysed one of these products and found it to be a tetrahydrohæmatoporphyrin. As the reduction proceeds, the solution becomes decolorized, the pigment being converted into a leuco-compound. If the solution be filtered at this stage and exposed to the air, the colourless leuco-derivative becomes rapidly oxidized to a pigment having the same spectrum as hæmatoporphyrin. In this connexion it is noteworthy that a large proportion of the hæmatoporphyrin, which is excreted in the urine in considerable amounts under certain pathological conditions, has been found to be present as a chromogen. Such urines darken markedly on standing owing to oxidation of the chromogen.

On more prolonged reduction at higher temperatures a substance is formed, having a spectrum resembling that of urobilin and giving a similar green fluorescence with zinc salts in ammoniacal solution. A substance having similar characters also results from the action of oxygen on a solution of hæmopyrrol. These two substances have not yet been proved to be identical.

A great deal of work on the products of the reduction of hæmin in acid solution has recently been carried out by Piloty, H. Fischer, and others, which, owing to lack of space, is not included in the foregoing account.

The products formed by the action of alkalies on oxyhæmoglobin are similar to those formed by acids, namely globin, hæmatin in alkaline solution, ammonia, and alkaline salts of certain fatty acids. The first product of the action of alkaline reducing agents,

hæmochromogen, has already been described. When hæmatin is heated with powerful alkaline reducing agents, such as zinc dust and caustic potash, the iron is split off, and a series of iron-free pigments, resembling hæmatoporphyrin are formed (Nobel and Rückert).

Oxidation of Hæmatin.—When hæmin, dissolved in glacial acetic acid, is oxidized by means of sodium bichromate, it yields two acids, which are soluble in ether, along with an amorphous substance containing iron, which is only soluble in alkalies, and some products, which are soluble in water (Küster). The two acids have the formulæ $C_8H_9NO_4$ and $C_8H_5O_5$. When the first is acted on by fixed alkalies, it loses ammonia and is converted into the second. When hæmatoporphyrin or phylloporphyrin is oxidized under the same conditions, they also yield those acids. A substance ($C_7H_9NO_2$) of closely allied nature is formed by the oxidation of hæmopyrrol (Küster).

Partial Syntheses of some of the Blood Pigments.—Laidlaw has shown that pure hæmatoporphyrin is converted into hæmochromogen, when it is dissolved in ammonia and reduced by hydrazine in the presence of a ferrous salt. He also isolated hæmatin from the mixture, and determined the percentage of iron in it. Zaleski has confirmed these observations, and has also prepared a substance resembling hæmatin from mesoporphyrine.

Further, Menzies has brought forward experimental evidence for a synthesis of hæmoglobin from hæmochromogen and globin. Ham and Balean have confirmed his results, using pure solutions of hæmochromogen and have shown that even when egg

albumin is used instead of globin, a pigment having the spectroscopic characters of hæmoglobin may still be synthesised. These observations indicate that the decomposition of hæmoglobin is, up to a certain point, a reversible process.

DISTRIBUTION OF HÆMOGLOBIN IN ANIMALS, MAINLY AFTER VON FÜRTH.

Echinodermata.	Vermes.	Mollusca.	Crustacea.	Other Arthropoda.	Tunicata.
Ophiactis virens, a species of Holothurian	*Chætopods* Lumbricus Lumbriculus Nais Chætogaster Enchytrachus Eunice Amphytrite (C.A.M.) Cirrhatulus Nereis Teretella Tubifex Arenicola Limnodrilus Glycera Capitella Aphrodite *Gephyreans* Phoronis Thalassema Hamingia *Nemertians* Polia Drepanaphorus Amphiporus *Hirudineæ* Nephelis Hirudo Hæmopis	Planorbis cornuus Arca tetragona Arca trapezia Arca barbata Arca noe ? Cardita aculeata Solen legumen Poromya granulata Tellina planata Capsa fragilis Peotinecalulus glycinurus *Lamellibranchs* Mytilus Anodonta Unio Mya Pecten	*Branchiopoda* Daphnia ? Apus Artemia Branchipus *Ostracods* Cypris ? *Copepods* Lernanthropus Clavella Congerioola	Chironomus (Larva)	None reported

DISTRIBUTION OF RESPIRATORY PIGMENTS OTHER THAN HÆMOGLOBIN, AFTER VON FÜRTH.

Echinoderms.	Vermes.	Mollusca.	Crustacea.	Other Arthropoda.
		HÆMOCYANIN.		
None	None	*Gastropods* Helix Limæmus Arion Fissurella Paludina Haliotis Turbo Murex Cassidaria Triton Cyclostoma Scaphander *Cephalopods* Octopus Sepia Eledone Loligo	*Decapods* Astacus Nephrops Palinurus Maia Cancer Carcinus Eryphia Callinectis	Spiders Epeira Tegmaria Pholeus
		ECHINOCHROME.		
Echinids Sphærechinus Echinus Strongyloccu-liotus	None	None	None	None
		CHLOROCRUORIN.		
None	*Chætopods* Sabella Siphonostoma Chloronema Branchionema Spirographis	None	None	None
		HÆMERYTHRIN.		
None	Gephyreans Phascolosoma Sipunculus	None	None	None
		ACHROGLOBIN.		
None	None	*Lamellibranchs* Pinna *Gastropods* Patella Chiton Doris	None	Ascidian

CHAPTER V.

THE HISTOHÆMATINS AND MYOHÆMATIN.

The Histohæmatins and Myohæmatin have not found their way into text-books because they do not belong to the ordinary pigments.

They are not derivatives of hæmoglobin because they occur in animals in which no hæmoglobin occurs. If one opens the thorax of an insect and takes out the alar muscle, puts it into a "compressorium," especially after clearing it with glycerol, and examines it with a spectroscope, a new and unfamiliar appearance presents itself (Fig. 11).

Fig. 11.—Spectrum of crushed insect's muscle.

From sponges up to man these immature products resembling hæmoglobin occur. The late Dr. Sorby laid stress on this point.

Unfortunately there is no method known by which this pigment can be isolated and its presence is merely inferred from its spectrum. However, it has a very wide distribution in the animal kingdom.

Sometimes it occurs in the form of "modified myohæmatin" whose spectrum is like that of hæmo-

chromogen but bands occur in a different position, and are much narrower and sharper (Fig. 12).

A good deal of discussion has taken place over this pigment, and the name of Hoppe-Seyler has prevented the acceptance of the writer's views.[1] The chemical position is undoubtedly weak, but doubtless in time this pigment will find its way into the text-books.

Once and for all, it is not a derivative of hæmoglobin, it is on its way in the process of evolution to become a hæmoglobin.

To show the spectrum of myohæmatin, the breast-muscle of the pigeon is well adapted after having been

Fig. 12.—Oxymyohæmatin; pigeon's muscle cleared with glycerol.

cleared with glycerol. A good light is required and an Abbe or other sub-stage condenser is a help.

The Histohæmatins have a universal distribution from sponges to man.

In Musca vomitoria the bands are as follow:—

1st band λ 615 to 593
2nd ,, λ 567·5 to 561·5
3rd ,, λ 554·5 to 546
4th ,, λ 532 to 511·5

These pigments are respiratory, being changed by oxidation and reduction as hæmoglobin is.

To sum up this question of Myohæmatin, it is an immature pigment on its way to form hæmoglobin,

[1] "Zeit. f. physiol. Chem.," 1889 and 1890.

CHAPTER VI.

QUANTITATIVE SPECTRUM ANALYSIS; THE SPECTROPHOTOMETER.

ANYONE attacking this subject must understand something about logarithms. Chambers's well-known tables may be used. Other special tables are published much cheaper, but to save trouble I have copied the tables (pp. 84-6) given by the Brothers Krüss in their book which is indispensable to the student of this subject.

Vierordt laid the foundation of this method, based on the researches of Roscoe and Bunsen. Hüfner has extended Vierordt's work and invented a new spectrophotometer which in an improved form is made by Hilger (Fig. 13).

Spectrophotometric Theory and Constants.—The unaided eye is unable to judge of the intensity of the absorption-bands or their breadth, and in using the spectrophotometer it is unwise to take many measurements without intervals of rest between each, because the retina gets tired. The eye should be shaded, and the eye-lids shut after each observation (see Screen in Fig. 15).

To quote Gamgee: "When, however, we are made acquainted with the remarkable and far-reaching conclusions which can be legitimately drawn from an accurate determination of the percentage of light of a

definite wave-length absorbed by colouring matters existing in solution, the beauty and the importance of the method of spectrophotometry become apparent". It was by means of the spectrophotometer that I was able to prove the important fact that entero-chlorophyll is a direct food-product (see Fig. 14). Its extraordinary persistence in the so-called liver of Invertebrates led me to think that it was of animal origin, but the spectrophotometer solved the mystery, and Dastre, approaching the matter from another side, by

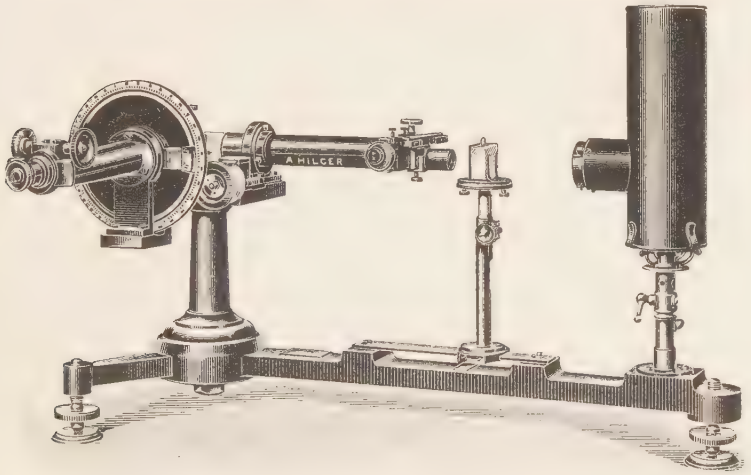


FIG. 13.—Hüfner's spectrophotometer.

feeding experiments on snails, proved the point. The data for the curve are given at the top of the page. "Until Vierordt's discovery, those coloured bodies whose visible spectrum presented no definite absorption-bands, were held to be beyond the scope of spectroscopic research" (Gamgee). It is a method surpassing all others in accuracy, permitting, in certain cases, of the accurate determination of data not to be ascertained in any other way.

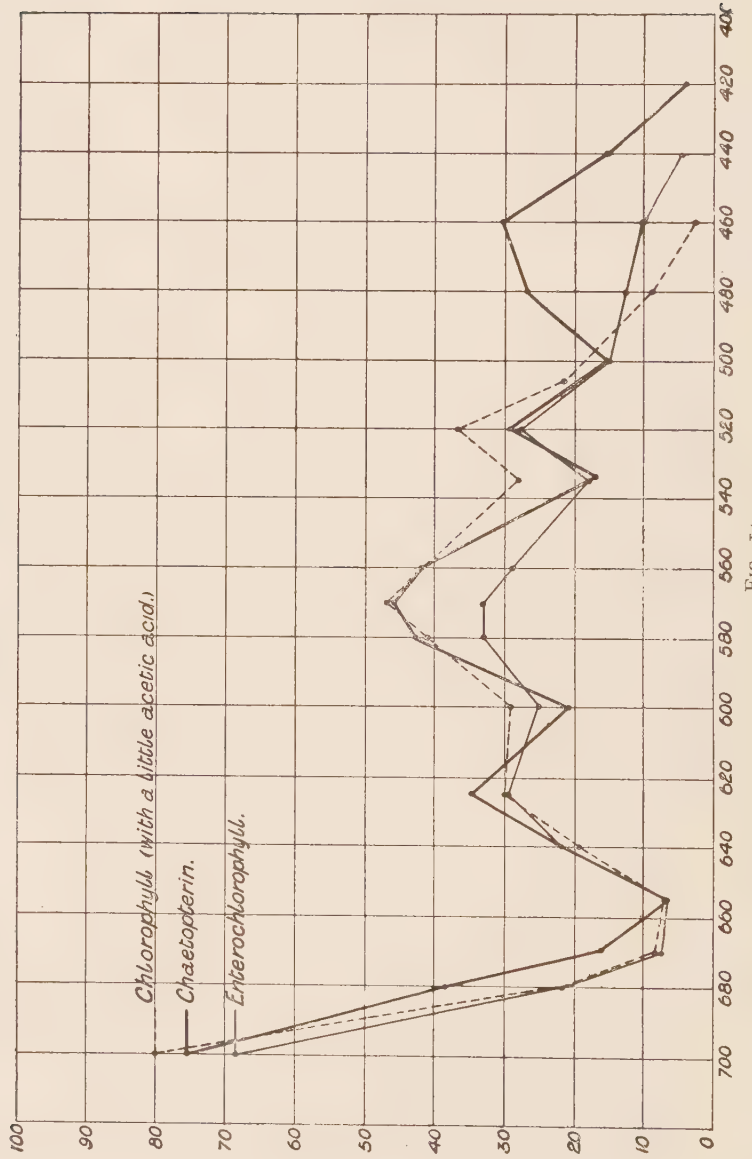

Chlorophyll (with a little acetic acid.)
Chaetopterin.
Enterochlorophyll.
100
90
80
70
60
50
40
30
20
10
0
700
680
660
640
620
600
580
560
540
520
500
480
460
440
420
400

FIG. 14.

The writer's spectrophotometer is, indeed, based on an improvement on Krüss's instrument which is an improved form of Vierordt's original one. The improvements were carried out by the late Mr. Otto Hilger who was skilled in the manufacture of screws, having been for years engaged in making such for a dividing-engine. In this form of instrument everything depends on the screws.

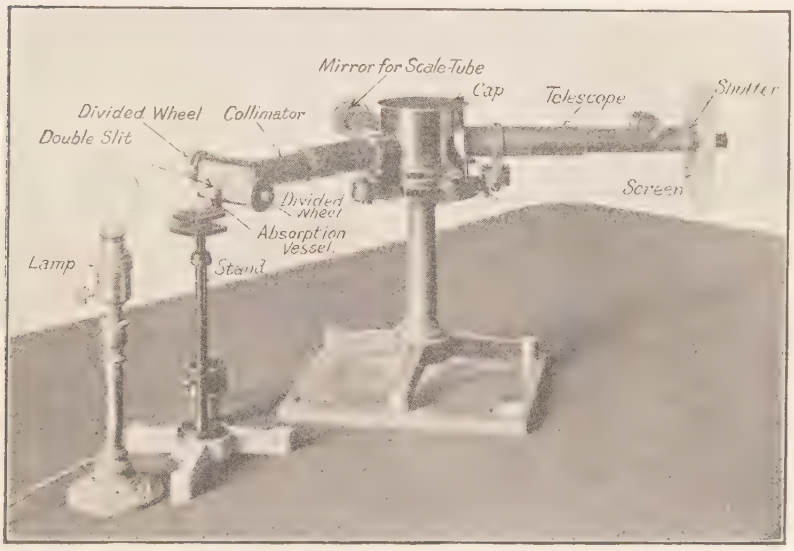


FIG. 15.—Dr. MacMunn's spectrophotometer.

The instrument is shown in the accompanying Fig. 15. I now proceed to describe it. It consists of the following parts: (1) The spectroscope—a single-prism "Chemical" Spectroscope, with collimator and observing telescope, etc. The prism is covered by a cap to exclude extraneous light. (2) The Scale-Tube, illuminated by a mirror as in the case of the spectroscope figured on page 10. (3) The double slit with platino-iridium or quartz jaws is so constructed as to

allow of symmetrical movement of the edges of the slit with reference to the middle line. (4) The blackened screen to protect the observing eye. (5) The wheel divided into 200 parts. (6) The Stand (Stativ) carrying the absorption vessel, with its Schulz rectangular block of glass ("Glaskörper"). This is 11 mm. from back to front and the Glaskörper is 10 mm. thick. Thus one can compare a depth of 1 mm. with one of 11 mm. This is the same as comparing a depth of 10 mm. with that of nothing.

The "Glaskörper" (or glass cube) enables one to compare the two spectra more accurately, as by proper levelling, provided by the levelling screws, a sharp line separates the spectra.

Then there is (7) the lamp. I now use an Auer-Welsbach incandescent lamp. It has the advantage of giving a more brilliant light and one free from the irritating rays (ultra violet) of short wave-length.

I know that Vierordt's method has been adversely criticized, but with this instrument, as described and figured, the error with due precaution is not more than 0·5 per cent. However, the book of the Brothers Krüss discusses all the methods of Spectrophotometry.

Fig. 16.—Morath shutter eye-piece.

Another piece of apparatus indispensable to the method of spectrophotometry is the Morath shutter in the eye-piece (Fig. 16). That here shown

is more elaborate than the one used by me, as no measuring apparatus is required with it. By it any part of the spectrum can be isolated, and I find a breadth of 10 mm. is best.

How can one isolate such a strip of spectrum ?

By means of the arbitrary scale and the interpolation curve, any part of the spectrum can be cut off.

To prevent worry in taking the readings of the divided wheel, and to obviate the necessity of going to take the readings, I have placed a concave mirror with the divided wheel in its focus. Thus, divisions of the wheel are magnified, and the observer sits quietly at the eye-end of the observing telescope. The numbers on the divided wheel are reversed, but one can allow for that.

Definition of the Extinction Coefficients.—This is the reciprocal of the number expressing the width of the stratum of a given medium required to reduce the intensity of light passed through it to one-tenth of its initial value. Suppose a solution of a colouring matter of a definite strength has a definite thickness of layer, equal to d, and is capable of reducing the intensity of light to one-tenth of its original value, then :—

The reciprocal of $d = \frac{1}{d}$; and if by E we represent the Extinction Coefficient, then

$$E = \frac{1}{d}.$$

The method of spectrophotometry rests on the determination of E for certain limited regions of the spectrum.

The formula for E that is usually employed in

spectrophotometric work is $E = -\log I'$, in which I' is the residual light remaining after passage through the solution of pigment placed in the absorption vessel already described. Reference may be made to Krüss's book for a proof of the formula. The following example is given to illustrate its application to a particular case.

Suppose that passing through a layer of coloured solution 1 cm. wide, the intensity of the light has been reduced to two-thirds of its original value, then—

$$E = -\log \tfrac{2}{3} = \log 3 - \log 2$$
$$= 0{\cdot}176091$$

The next term to define is the *Absorption Relation.*

Let C and C′ represent the concentration of two coloured solutions of which E and E′ are the Extinction Coefficients, then

$$C : E \text{ as } C' : E'$$
$$\text{and } \therefore \quad \frac{C}{E} = \frac{C'}{E'} = A$$

that is A, the Absorption Relation, is the relation of the concentration of a coloured solution to its Extinction Coefficient.

Having once determined the value of A for a given colouring matter, we can always find the amount of that colouring matter present in a solution of unknown strength. We determine C′ by this equation :—

$$C' = A \times E'.$$

This value A is generally determined for two spectral regions. The two methods, as already stated, are those of Hüfner and Vierordt. With the perfected instrument described above, I believe Vierordt's method is as good as that of Hüfner.

In Germany the modified spectrophotometer of

König has been used, but as Marchlewski observes: "The application of the Martenskönig's apparatus is not plain sailing as is unfortunately but too evident".

The following data according to Krüss are indispensable in connexion with this work. Let us call the Absorption Relations of oxyhæmoglobin Ao and A′o; and for reduced hæmoglobin Ar and A′r; for methæmoglobin Am and A′m; for carbon monoxide hæmoglobin Ac and A′c respectively.

Hüfner found the following constants for oxyhæmoglobin :—

Amount of O_2 Hb in grams in 1 c.c. of solution	Ao	A′o
0·0010514	0·001430	0·001128
0·007812	0·001435	0·001114
Mean	0·001452	0·001110

Von Noorden concluded that the following are the Absorption Relationships of O_2Hb :—

$$Ao = 0{\cdot}001330$$
$$A'o = 0{\cdot}001000$$

I have found these numbers accurate, and have arrived at the same numbers for the two regions of the spectrum, namely 14 and 13·99 per cent in the case of my own blood.

In the case of reduced hæmoglobin Hüfner found :—

$$Ar = 0{\cdot}001091$$
$$A'r = 0{\cdot}001351$$
Mean $= 0{\cdot}001351$ for Ar
,, $= 0{\cdot}001499$ for A′r

For Methæmoglobin the following are the constants found by Otto and Hüfner :—

$$A_m = 0{\cdot}002602$$
$$A'_m = 0{\cdot}001990$$

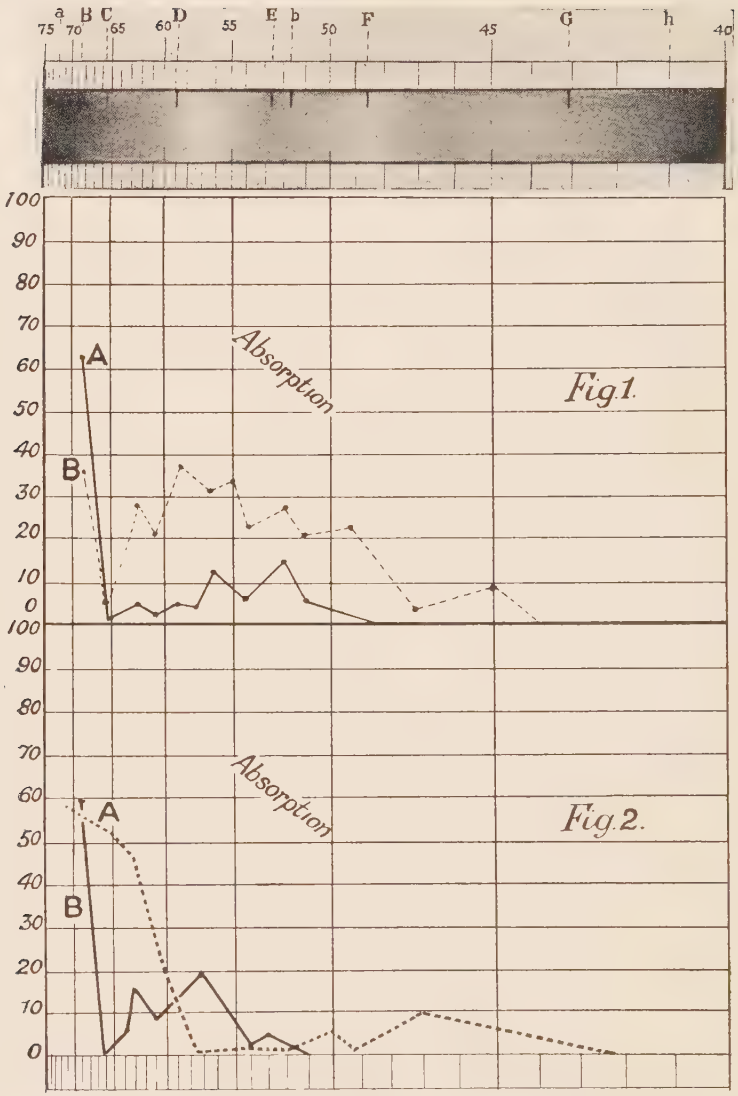


CHART II.

FIG. 1.—Chlorophyll alcohol 90 per cent. *Lupinus*. A. Strong solution. B. Weak solution.

FIG. 2.—Chlorophyll of *Fucus* in alcohol 90 per cent. and of a *red* alga. Phycoerythrin spectrophotometric curve *red* = A. Chlorophyll = B. Curve green.

The indispensable book of the Brothers Krüss must be consulted for carbon-monoxide-hæmoglobin, bilirubin, hydrobilirubin and other bile-pigments.

The curves given in Fig. 14 show those of chlorophyll, chætoptorin and entero-chlorophyll. The accompanying Chart II. shows on a smaller scale those of the chlorophyll of Lupinus in 90 per cent alcohol (Fig. 1), A and B being respectively stronger and weaker solutions; and of the chlorophyll of Fucus (Fig. 2) and the red pigment phycoerythrin (A) which occurs in all the red Phycophyceæ.

The same point was not taken for Fig. 1 A and B at λλ 59, which accounts for the discrepancy in the curve.

Special table of the Extinction Coefficients of the unabsorbed light.[1]

The data given are from 0·999 to 0·001, i.e. from 99 to 1 of the divided wheel of the spectrophotometer.

[1] After the Brothers Krüss.

Strength Light.	Coefficient Extinction.	Strength Light.	Coefficient Extinction.	Strength Light.	Coefficient Extinction.
0·990	0·00437	0·645	0·19045	0·473	0·32514
0·980	0·00878	0·640	0·19382	0·470	0·32791
0·970	0·01323	0·635	0·19723	0·468	0·32976
0·960	0·01773	0·630	0·20066	0·465	0·33255
0·950	0·0228	0·625	0·20412	0·463	0·33442
0·940	0·02688	0·620	0·20762	0·460	0·33725
0·930	0·03152	0·615	0·21113	0·458	0·33914
0·920	0·03622	0·610	0·21468	0·455	0·34199
0·910	0·04096	0·605	0·21825	0·453	0·34391
0·900	0·04576	0·600	0·22185	0·450	0·34679
0·895	0·04818	0·598	0·22330	0·448	0·34873
0·890	0·05061	0·595	0·22549	0·445	0·35164
0·885	0·05306	0·593	0·22695	0·443	0·35360
0·880	0·05552	0·590	0·22915	0·440	0·35655
0·875	0·05800	0·588	0·23063	0·438	0·35853
0·870	0·06049	0·585	0·23285	0·435	0·36152
0·865	0·06299	0·583	0·23434	0·433	0·36352
0·860	0·06551	0·580	0·23658	0·430	0·36654
0·855	0·06804	0·578	0·23808	0·428	0·36856
0·850	0·07059	0·575	0·24034	0·425	0·37162
0·845	0·07315	0·573	0·24185	0·423	0·37366
0·840	0·07573	0·570	0·24413	0·420	0·37676
0·835	0·07832	0·568	0·24566	0·418	0·37883
0·830	0·08093	0·565	0·24796	0·415	0·38196
0·825	0·08355	0·563	0·24950	0·413	0·38405
0·820	0·08619	0·560	0·25182	0·410	0·38722
0·815	0·08885	0·558	0·25337	0·408	0·38934
0·810	0·09152	0·555	0·25571	0·405	0·39255
0·805	0·09421	0·553	0·25728	0·403	0·39260
0·800	0·09691	0·550	0·25964	0·400	0·39794
0·795	0·09964	0·548	0·26122	0·399	0·39903
0·790	0·10238	0·545	0·26361	0·398	0·40012
0·785	0·10514	0·543	0·26521	0·397	0·40121
0·780	0·10791	0·540	0·26761	0·396	0·40231
0·775	0·11070	0·538	0·26922	0·395	0·40341
0·770	0·11351	0·535	0·27165	0·394	0·40451
0·765	0·11634	0·533	0·27328	0·393	0·40561
0·760	0·11919	0·530	0·27573	0·392	0·40672
0·755	0·12206	0·528	0·27737	0·391	0·40783
0·750	0·12494	0·525	0·27985	0·390	0·40894
0·745	0·22785	0·523	0·28150	0·389	0·41006
0·740	0·13077	0·520	0·28400	0·388	0·41117
0·735	0·13372	0·518	0·28568	0·387	0·41229
0·730	0·13668	0·515	0·28820	0·386	0·41342
0·725	0·13967	0·513	0·28989	0·385	0·41454
0·720	0·14267	0·510	0·29243	0·384	0·41567
0·715	0·14570	0·508	0·29414	0·383	0·41681
0·710	0·14875	0·505	0·29671	0·382	0·41794
0·705	0·15182	0·503	0·29844	0·381	0·41908
0·700	0·15491	0·500	0·30103	0·380	0·42022
0·695	0·15802	0·498	0·30278	0·379	0·42137
0·690	0·16116	0·495	0·30540	0·378	0·42251
0·685	0·16431	0·493	0·30716	0·377	0·42366
0·680	0·16750	0·490	0·30981	0·376	0·42482
0·675	0·17070	0·488	0·31159	0·375	0·42597
0·670	0·17393	0·485	0·31426	0·374	0·42713
0·665	0 17718	0·483	0·31606	0·373	0·42830
0·660	0·18046	0·480	0·31876	0·372	0·42946
0·655	0·18376	0·478	0·32058	0·371	0·43063
0·650	0·18709	0·475	0·32331	0·370	0·43180

Strength Light.	Coefficient Extinction.	Strength Light.	Coefficient Extinction.	Strength Light.	Coefficient Extinction.
0·369	0·43298	0·310	0·50864	0·251	0·60033
0·368	0·43416	0·309	0·51005	0·250	0·60206
0·367	0·43534	0·308	0·51145	0·249	0·60381
0·366	0·43652	0·307	0·51287	0·248	0·60555
0·365	0·43771	0·306	0·51428	0·247	0·60731
0·364	0·43890	0·305	0·51571	0·246	0·60907
0·363	0·44010	0·304	0·51713	0·245	0·61084
0·362	0·44130	0·303	0·51856	0·244	0·61262
0·361	0·44250	0·302	0·52000	0·243	0·61440
0·360	0·44370	0·301	0·52144	0·242	0·61619
0·359	0·44491	0·300	0·52288	0·241	0·61799
0·358	0·44613	0·299	0·52433	0·240	0·61979
0·357	0·44734	0·298	0·52579	0·239	0·62161
0·356	0·44855	0·297	0·52726	0·238	0·62343
0·355	0·44978	0·296	0·52871	0·237	0·62526
0·354	0·45100	0·295	0·53018	0·236	0·62709
0·353	0·45223	0·294	0·53166	0·235	0·62894
0·352	0·45346	0·293	0·53314	0·234	0·63079
0·351	0·45470	0·292	0·53462	0·233	0·63265
0·350	0·45594	0·291	0·53611	0·232	0·63452
0·349	0·45718	0·290	0·53761	0·231	0·63639
0·348	0·45843	0·289	0·53919	0·230	0·63828
0·347	0·45968	0·288	0·54061	0·229	0·64017
0·346	0·46093	0·287	0·54212	0·228	0·64207
0·345	0·46219	0·286	0·54364	0·227	0·64398
0·344	0·46345	0·285	0·54516	0·226	0·64900
0·343	0·46471	0·284	0·54669	0·225	0·64782
0·342	0·46598	0·283	0·54822	0·224	0·64976
0·341	0·46725	0·282	0·54976	0·223	0·65170
0·340	0·46853	0·281	0·55130	0·222	0·65365
0·339	0·46981	0·280	0·55285	0·221	0·65561
0·338	0·47109	0·279	0·55440	0·220	0·65758
0·337	0·47238	0·278	0·55596	0·219	0·65956
0·336	0·47367	0·277	0·55753	0·218	0·66155
0·335	0·47496	0·276	0·55190	0·217	0·66315
0·334	0·47626	0·275	0·56067	0·216	0·66555
0·333	0·47756	0·274	0·56225	0·215	0·66757
0·332	0·47887	0·273	0·56384	0·214	0·66959
0·331	0·48018	0·272	0·56544	0·213	0·67163
0·330	0·48149	0·271	0·56704	0·212	0·67367
0·329	0·48281	0·270	0·56864	0·211	0·67572
0·328	0·48413	0·269	0·57025	0·210	0·67779
0·327	0·48546	0·268	0·57187	0·209	0·67986
0·326	0·48679	0·267	0·57349	0·208	0·68194
0·325	0·48812	0·266	0·57512	0·207	0·68403
0·324	0·48946	0·265	0·57676	0·206	0·68614
0·323	0·49080	0·264	0·57840	0·205	0·68825
0·322	0·49215	0·263	0·58005	0·204	0·69037
0·321	0·49350	0·262	0·58170	0·203	0·69251
0·320	0·49485	0·261	0·58336	0·202	0·69465
0·319	0·49621	0·260	0·58503	0·201	0·69681
0·318	0·49758	0·259	0·58671	0·200	0·69897
0·317	0·49895	0·258	0·58839	0·199	0·70115
0·316	0·50032	0·257	0·59007	0·198	0·70334
0·315	0·50169	0·256	0·59176	0·197	0·70554
0·314	0·50308	0·255	0·59346	0·196	0·70775
0·313	0·50446	0·254	0·59517	0·195	0·70995
0·312	0·50585	0·253	0·59688	0·194	0·71220
0·311	0·50724	0·252	0·59860	0·193	0·71445

Strength Light.	Coefficient Extinction.	Strength Light.	Coefficient Extinction.	Strength Light.	Coefficient Extinction.
0·192	0·71670	0·133	0·87615	0·074	1·13077
0·191	0·71897	0·132	0·87943	0·073	1·13668
0·190	0·72125	0·131	0·88273	0·072	1·14267
0·189	0·72354	0·130	0·88606	0·071	1·14875
0·188	0·72585	0·129	0·88942	0·070	1·15491
0·187	0·72816	0·128	0·89279	0·069	1·16116
0·186	0·73049	0·127	0·89620	0·068	1·16750
0·185	0·73283	0·126	0·89963	0·067	1·17393
0·184	0·73519	0·125	0·90309	0·066	1·18046
0·183	0·73755	0·124	0·90658	0·065	1·18709
0·182	0·73993	0·123	0·91010	0·064	1·19382
0·181	0·74233	0·122	0·91365	0·063	1·20066
0·180	0·74473	0·121	0·91722	0·062	1·20761
0·179	0·74715	0·120	0·92082	0·061	1·21468
0·178	0·74958	0·119	0·92446	0·060	1·22185
0·177	0·75423	0·118	0·92812	0·059	1·22915
0·176	0·75449	0·117	0·93182	0·058	1·23658
0·175	0·75697	0·116	0·93555	0·057	1·24413
0·174	0·75946	0·115	0·93931	0·056	1·25182
0·173	0·76196	0·114	0·94310	9·055	1·25964
0·172	0·76448	0·113	0·94693	0·054	1·26761
0·171	0·76701	0·112	0·95079	0·053	1·27573
0·170	0·76956	0·111	0·95468	0·052	1·28400
0·169	0·77212	0·110	0·95861	0·051	1·29243
0·168	0·77470	0·109	0·96258	0·050	1·30103
0·167	0·77729	0·108	0·96658	0·049	1·30981
0·166	0·77990	0·107	0·97602	0·048	1·31876
0·165	0·78252	0·106	0·97470	0·047	1·32791
0·164	0·78516	0·105	0·97882	0·046	1·33725
0·163	0·78782	0·104	0·98297	0·045	1·34679
0·162	0·79049	0·103	0·98719	0·044	1·35655
0·161	0·79318	0·102	0·99140	0·043	1·36654
0·160	0·79588	0·101	0·99568	0·042	1·37676
0·159	0·79861	0·100	1·00000	0·041	1·38722
0·158	0·8015	0·099	1·00437	0·040	1·39794
0·157	0·80411	0·098	1·00878	0·039	1·40894
0·156	0·80688	0·097	1·01323	0·038	1·42022
0·155	0·80967	0·096	1·01773	0·037	1·43180
0·154	0·81248	0·095	1·02228	0·036	1·44370
0·153	0·81531	0·094	1·02688	0·035	1·45594
0·152	0·81816	0·093	1·03152	0·034	1·46853
0·151	0·82103	0·092	1·03622	0·033	1·48149
0·150	0·82391	0·091	1·04096	0·032	1·49485
0·149	0·82682	0·090	1·04546	0·031	1·50864
0·148	0·82974	0·089	1·05061	0·030	1·52288
0·147	0·83269	0·088	1·05552	0·029	1·53761
0·146	0·83563	0·087	1·06094	0·028	1·55285
0·145	0·83864	0·086	1·06551	0·027	1·56864
0·144	0·84164	0·085	1·07059	0·026	1·58503
0·143	0·84467	0·084	1·07573	0·025	1·60206
0·142	0·84772	0·083	1·08093	0·024	1·61979
0·141	0·85079	0·082	1·08619	0·023	1·63828
0·140	0·85388	0·081	1·09152	0·022	1·65758
0·139	0·85699	0·080	1·09692	0·021	1·67779
0·138	0·86013	0·079	1·10238	0·020	1·69897
0·137	0·86328	0·078	1·10791	0·015	1·82391
0·136	0·86647	0·077	1·11351	0·010	2·00000
0·135	0·86967	0·076	1·11919	0·005	2·30103
0·134	0·87290	0·075	1·12494		

CHAPTER VII.

FLUORESCENCE AND PHOSPHORESCENCE.

The Fluoroscope.—To observe fluorescence a small box is required provided with a plano-convex lens of quartz. The general arrangement is shown in Fig. 17. The inside of the box is coated with dead black, and the stand supporting the vessel is also dead black. The microspectroscope is that of Zeiss provided with

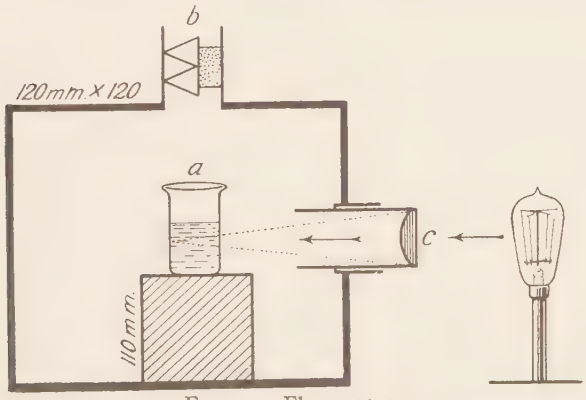


FIG. 17.—Fluoroscope.

a wave-length scale. The lens C is capable of being focussed on the vessel A.

This simple apparatus was made for me by Messrs. Baker of High Holborn and it answered admirably. The vessel A is made of quartz. The direct vision spectroscope cuts off the ultra-violet rays.

The whole question of fluorescence and of phosphorescence is discussed by Lommel and by Glazebrook in

their respective textbooks. Sir George Stokes laid the foundations of this interesting subject. The fluorescence of sulphate of quinine is due to the extreme violet rays. In chlorophyll, however, the greater part of the effect is produced by the visible light of the spectrum and begins in the red end of the spectrum near the line B. It will be remembered that the absorption spectrum of chlorophyll has a strongly marked dark band between B and C.

Quartz has the power of transmitting the ultra-violet rays far more perfectly than has glass, and hence the necessity of using a quartz lens in the fluoroscope. Naphthalin-red fluoresces with orange-yellow tints of unusual brilliancy, and its fluorescent-spectrum is complementary to that of its absorption-spectrum, for every dark band in the absorption-spectrum corresponds to a bright band in the fluorescing spectrum.

Æsculin is a substance contained in the bark of the horse-chestnut which shows a most remarkable sky-blue fluorescence. The peculiarity of fluorescence was first observed in the case of a spar—fluor spar—from which the word fluorescence is derived. In calcium fluoride it is also well marked. According to Lommel, if the light before it reaches the lens be allowed to pass through just such another solution of æsculin, contained in a glass vessel with parallel walls, it no longer presents a blue shimmer but becomes scarcely perceptible.

Phosphorescence is also an effect of absorbed light. According to Lommel, phosphorescence may be described as fluorescence which is prolonged for a certain length of time beyond the action of the

exciting rays. We saw how phosphorescence is the light of the deep sea, and how fluorescence helps sea-weeds by the agency of their reinforcing pigments to absorb rays which otherwise would be lost to them. Many fungi and bacteria emit phosphorescent light. It disappears in an atmosphere devoid of oxygen, and reappears on the admission of free oxygen. Hence, as Beyerinck says, "the phosphorescent bacteria afford a delicate test for the activity of assimilation". All conditions which facilitate respiration intensify phosphorescence. As far as we can gather from the results of investigations into phosphorescence in the case of animals, it does not differ in principle from the phosphorescence of plants and it does not seem to be directly dependent upon the respiratory processes.

The so-called phosphorescence of the moss Schistega is not phosphorescence at all, but is produced solely by the reflection of the daylight from peculiarly formed cells, says Strasburger. That a plant should develop eyes or at least lenses to make up for the want of light in caves, is a beautiful fact in adaptation.

CHAPTER VIII.

THE PIGMENTS OF VERTEBRATE AND INVERTEBRATE BILE, AND OF THE URINE.

We consider these together, for although there is no bile in invertebrates, yet some pigments, for instance reduced hæmatin (hæmochromogen), is found both in the Vertebrates and in the Invertebrates, as has been shown by Dastre and Floresco. It is now proved that hæmatoidin and bilirubin are identical. Bilirubin has the formula $C_{16}H_{18}N_2O_3$, or according to Orndorff and Teeple $C_{32}H_{36}N_4O_6$. It is usually amorphous, but can be crystallized (Fig. 10). The crystals vary in colour, sometimes they are yellowish-red, sometimes reddish-brown, sometimes more truly red. The crystals can be obtained by allowing a chloroformic solution to evaporate at the air temperature. The crystals are rhombic plates with obtuse angles which are often rounded. Bilirubin is insoluble in water, it behaves like an acid, but when it occurs in animal fluids it is as alkali-bilirubin. It is slightly soluble in alcohol, ether, benzene, carbon disulphide, amyl alcohol, oils and glycerol. In hot chloroform it is more soluble than in cold. Bilirubin gives no absorption-bands, but it absorbs strongly the violet end of the spectrum.

Gmelin's Reaction.—As carried out with strong, impure nitric acid, an aqueous solution of alkali-bilirubin gives a play of colours at the junction of the liquids

in the following order from above down : green, blue, violet, red, reddish-yellow. The end reaction corresponds to choletelin. The test is said to be so delicate as to detect the presence of one part of bilirubin in 80,000 parts of liquid. If the nitric acid is too strong, the play of colours occurs too quickly, so that it is best to dilute the acid.

The first oxidation step in this reaction is the forma-

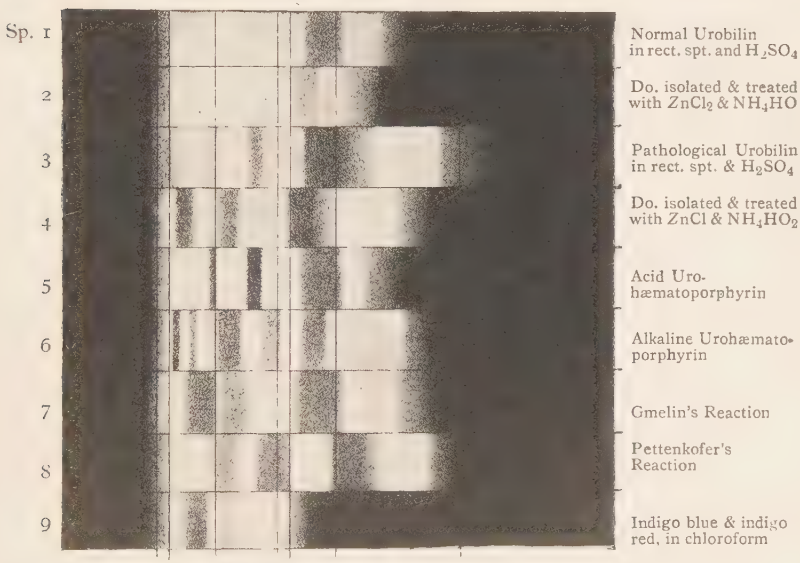


CHART III.

tion of biliverdin, then follows the bilicyanin of Heynsius and Campbell, and the final step is choletelin (Maly). This last pigment in acid solution gives a band between *b* and F. Bilirubin is best estimated quantitatively by the spectrophotometer (Chart III., Spectrum 7).

Biliverdin.—$C_{16}H_{18}N_2O_4$ or twice these numbers. It is formed by the oxidation of bilirubin, and accord-

ing to some occurs in the placenta of the bitch, in the egg-shells of birds and in other places. I have found it in hydrocele fluid into which blood had been extravasated.

It is an amorphous pigment and has not yet been crystallized. It is insoluble in water, chloroform and ether; but it is soluble in alcohol, glacial acetic acid and in alkalies, in which it forms a brownish-green solution. According to the researches of Haycraft and Scofield, biliverdin may be reduced to bilirubin.

The Bile of Invertebrates.—The "bile" of Invertebrates differs from that of Vertebrates in having no bile salts and no bile pigments. The principal colouring matter is entero-chlorophyll which, before the use of the spectroscope, was mistaken for biliverdin. Hæmochromogen is also present both in Vertebrates and in Invertebrates. Dastre and Floresco have contributed most to our knowledge of the chemistry of bile. These investigators have studied this subject very thoroughly, and have concluded that bilirubin does not exist in the bile of vertebrates as such, but in combination with sodium as a neutral bilirubinate. Biliverdin, on the other hand, they say exists in the acid condition. The bilirubinates are less soluble in water than the alkaline biliverdinates. These authors state that in addition to these pigments there are present in the bile of the gall-bladder under normal conditions two other pigments of a biliprasin nature. One, a yellow brown one, biliprasinate of sodium, and the other a biliverdinate which is decolorized under the action of light. These authors also refer to a hepatic iron and attribute a "fonction martiale" to the liver. They

have employed a quantitative method for the determination of iron in the liver which is colorimetric. The spectrophotometer is the only instrument that can be relied on. They applied a similar method to the invertebrate liver and to the tissues of crustaceans and molluscs. They come to some important conclusions for which the original memoir should be consulted. This treatise is illustrated by a coloured plate representing the spectra of many pigments.

Urochrome.—This is the normal pigment of the urine. I use this term in Dr. Garrod's sense, not in that of the late Dr. Thudichum. Urochrome absorbs the violet part of the spectrum but gives no absorption-bands. It is convertible, apparently, into a urobilin-like body. It is nitrogenous, but contains no iron. Riva maintains that urobilin on careful oxidation with permanganate of potash yields a body similar to urochrome. According to Garrod, urochrome may be detected by its transformation into urobilin. The method of preparing urochrome is rather complicated, as it is very easily decomposed by acids. Klemperer has estimated urochrome by a colorimetric method, but it is best estimated by the spectrophotometer.

Urobilin.—This was first isolated by Jaffé. It has a beautiful green fluorescence when the urine is treated with zinc chloride and ammonia (Chart III., Spectrum 2). Different urobilins have been described and some of them named febrile or pathological. Hammarsten remarks: "the possibility of the occurrence of different urobilins in the urine cannot be denied; but as urobilin is a readily changeable body and difficult to purify from other urinary pigments, the

question as to the occurrence of different urobilins must still be considered open".

Normal urobilin can only be detected in normal urine in which it exists as a chromogen. It is transformed to urobilin by exposure to the air, by adding an acid or by adding an oxidizing agent; but it plays a very small, if any, part in the coloration of urine. That choletelin and normal urobilin are closely connected appears from the work of Disqué and of Stokvis. I obtained a body closely resembling choletelin by oxidizing acid hæmatin with peroxide of hydrogen. It has been shown by Garrod and Hopkins that urobilin and hydrobilirubin are not identical, since their percentage compositions are quite different, thus :—

Hydrobilirubin has C, 64·8 per cent ; H, 6·93 per cent ; N, 9·22 per cent ; while

Urobilin has C, 63·46 per cent ; H, 7·67 per cent ; N, 4·09 per cent.

According to these authors, stercobilin has the same composition as urinary urobilin, namely 4·17 per cent of nitrogen.[1]

Urobilin is absent from the fæces of infants and from them after taking calomel and in enteric fever, the reason apparently being that no putrefaction occurs under these conditions. There is also no stercobilin under these circumstances. Various methods have been devised for precipitating this pigment from urine, but these need not be discussed here.

Uroerythrin.—This is not a normal constituent of urine. It gives a red colour to deposits of urates, the

[1] Cf. Hammarsten, p. 603.

so-called sedimentum lateritium. The pigment has been studied by Zoja, Riva and Garrod. Solutions have strong absorption beginning between B and E and extending close to F, and have two absorption-bands connected by a shading. This spectrum was first discovered by me. Alkalies change the colour of the pigment when in the solid state to a grass-green. According to Porcher and Hervieux, uroerythrin is a scatol pigment. Scatol and indol belong to the so-called aromatic substances which are produced mainly in the intestine.

Indoxyl Compounds.—Indoxyl is a product of the oxidation of indol, which latter may arise from sources other than the intestine, for instance in putrid empyemata and in other foci of putrefaction. The name indican was given by the late Dr. Schunck to the pigment obtained from a plant, Isatis tinctoria. Indican is a glucoside, but the pigment in the urine derived from indoxyl although called urinary indican, is not a glucoside. Urinary indican is indoxyl-sulphate of potassium. Some of the indoxyl is conjugated with glycuronic acid, and this indoxyl-glycuronic acid forms unstable salts. As no putrefactive processes take place in the intestines of newly-born infants owing to the absence of bacteria, there is no indoxyl either in their intestine or in their urine. In normal human urine in the adult, only from 1 to 6 milligrams of indican occur in 1000 c.c. The large ingestion of meat increases the quantity.

Detection and Estimation of Indoxyl Sulphate of Potassium.—The acid must be separated from the base, and indigo red, as well as indigo blue, may be set free and detected spectroscopically. In Chart III.,

Spectrum 9, the darker band is that of indigo blue, that after D is indigo red.

The method of obtaining the coloured pigment from its chromogen is due to Jaffé. Dilute bleaching powder (calcium hypochlorite), or peroxide of hydrogen, or Obermayer's reagent is added to the urine, and, when cool, the mixture is shaken with chloroform and allowed to settle, when it is found that the chloroform has taken up the pigment. Obermayer's reagent is "a two per one thousand of ferric chloride in concentrated hydrochloric acid". Excess of the oxidizing agent must be avoided, hence the bleaching salt must be diluted and added gradually or else the colour will be destroyed. The test-tube should be inverted after each addition of the chloroform to avoid an emulsion forming. By using Obermayer's method the substances causing an emulsion are removed.

Scatoxyl Compounds.—Scatoxyl is an oxidation product of scatol and arises under similar conditions as does indol, namely those of putrefaction. It appears in the urine in two forms: (1) mainly as potassium scatoxyl-sulphate, and (2) as a salt of scatoxyl-glycuronic acid. To detect scatoxyl, equal volumes of urine and hydrochloric acid are put into a test-tube with a little amyl alcohol and some weak bleaching-powder or a fragment of potassium chlorate, and the vessel repeatedly inverted. The urine becomes reddish and the amyl alcohol rosy-red. Rössler states that scatoxyl-red gives an absorption-band between C and D.[1] The extract when shaken with an alkali becomes pale yellow.

[1] Cf. Dixon Mann, p. 173.

Urorosein.—This was first discovered in urine by Nencki and Sieber. It occurs also as a chromogen which only becomes coloured on adding an oxidizing agent. It is insoluble in ether, chloroform and benzene, but soluble in water, amyl alcohol and acidulated ethyl-alcohol. It can be extracted from urine only by amyl-alcohol. It is unstable and gives a characteristic spectrum, namely an absorption-band nearly midway between D and E. It may be present in healthy individuals. Obermayer's method, already referred to, may be used for the detection of urorosein. Both scatoxyl and this pigment are separated from urine by amyl alcohol, but the separation of the one from the other is not easy. Rössler says that the spectrum of scatoxyl-red consists of a band between C and D, but according to Stokvis of two bands between D and E. According to Huppert[1] the best method of detecting urorosein is to obtain the pigment in the solid form and find whether it yields scatol after treatment with zinc dust.

Alkaptonuria.—This is an unusual condition of the urine, and from the spectroscopic point of view is of no importance, as alkapton yields no absorption-bands.

Melanuria.—Melanin sometimes appears in the urine, but it also yields no absorption-bands.

Blood-pigment in the Urine.—This is best detected by the spectroscope. If it occurs in the soluble state, it is easily detected by this instrument, but the guaiacum and peroxide of hydrogen test is open to several objections. If it is in the insoluble state and clinging to the urates, these should be filtered off and

[1] Cf. Neubauer and Vogel, 1898, and Mann, p. 176.

extracted with alcohol and ammonia; to the extract, sulphide of ammonium should be added when the bands of hæmochromogen should appear, as I first showed.

In hæmaturia, as a rule, we find the bands of hæmoglobin or of methæmoglobin. In intermittent hæmoglobinuria we generally find the spectrum of the latter. The centrifuge facilitates the separation of sediments.

Bile-pigments in the Urine.—Bilirubin and biliverdin are the pigments most usually found. Bilicyanin is the pigment which corresponds to the blue zone in Gmelin's test (Chart III., Spectrum 7). Pettenkofer's test gives a well-marked spectrum; this is, however, unreliable, but if the bile-salts are isolated from urine, Spectrum 7 of Chart III. is obtained.

Extraneous Pigmentary Substances in Urine.—For malingering purposes, people often add various pigments to their urine, for instance methylene blue or methyl blue, tea, etc. All these are detectable. Eosine, methylene blue, etc., give well-marked bands. Eosine is used to colour sweets, and I have known the colour of eosine in urine a puzzle to certain physicians. Methylene blue was used by a prisoner who wanted to get into hospital to escape prison discipline. There are various drugs which colour the urine, such as iodine, salicylic acid, guaiacol, izal, phenacetin, antipyrin, rhubarb, etc.

CHAPTER IX.

INVERTEBRATE PIGMENTS GENERALLY.

The best classification of these is that of von Fürth. He divides them into: (1) Those of the tissues, (2) respiratory pigments, (3) Those of the excretions.

Pigments of the Tissues.—Von Fürth first discusses the significance of chlorophyll in the animal kingdom and lays stress on Engelmann's observations on Vorticelleæ.

Pigments of Protozoa.—The experiments of Reinke and Rodenwald on the Myxomycetæ, which form a blue-black pigment on oxidation, are our source of information. Even the Lipochromes are not wanting, as Euglena sanguinea is an example (von Wittich). Sir E. Ray Lankester found a blue pigment in Stentor cœruleus. Its spectrum consists of two bands, one in the red before C, and the other in the green between D and E. This pigment was named by its discoverer, blue stentorin.

Pigments of Sponges.—Most of the yellow and red pigments of sponges are lipochromes such as in Tedania and Papilina. I have found a black pigment in an Antarctic sponge which has not yet been identified.

Pigments of Cœlenterates.—Most of these have still to be examined, although the late Professor Krukenberg, Professor Mackendrick and myself worked at the subject. The medusæ contain a blue

pigment which is soluble in distilled water and becomes rose-red on warming, while at the boiling-point the colour vanishes (Krukenberg). The blue solution has a fine red fluorescence; it gives three absorption-bands: (1) in the orange yellow between C and D; (2) in the yellow band after D; and (3) in the green blue (Platt). Another pigment of the Medusæ is known as Pelagein, a violet pigment obtained from Pelagia.

Pigments of Blue Corals.—To Moseley and Liversedge we owe our first observations on the blue coral, Heliopora cœrulea; it is apparently a unique substance. I have examined a great number of Actiniæ and Corals. In the former I found a kind of hæmatin and also confirmed Moseley's statement as to the occurrence of polyperythrin in the former. I found that this pigment polyperythrin is apparently identical with hæmatoporphyrin, although the apparent identity rests only on spectroscopic evidence at present.

Moseley's Actinochrome.—This is a widely distributed pigment; its spectrum differs from hæmochromogen. In the mesoderm of many sea-anemones I have found biliverdin as well as hæmochromogen; the former gave Gmelin's reaction.

In Cerianthus membranaceus, Krukenberg found a purple pigment, Purpuridin, but it gave no absorption-band. The same author describes Floridines among rose and purple coloured corals. Many red pigments of the Cœlenterates according to Minkowski and Krukenberg belong to the lipochromes, but they possess no respiratory properties; such occur in Tubularia, Pennaria, etc.

In the work "The Fauna of the Maldive and Laccadive Archipelagos," we have described the

pigments of Cœnopsammia, of Dendrophyllia remea, Heliopara cœrulea, and remarks on the coral pigments. For the importance of chlorophylloid pigments in Corals, see Hickson's "Naturalist in North Celebes".

The Pigments of Echinoderms.—The Echinoderms owe their colour mainly to lipochrome, but I have also found a hæmatoporphyrin which is Moseley's polyperythrin in some of them. Lipochromes occur in the integument of Astropecten aurantiacus, in Asterias rubens, etc. Lipochromes also occur in Holothurians, e.g. in Holothuria nigra and in Holothuria poli.

Pigments of other Echinoderms.—Antedonin was first described by Moseley in Antedon rosaceus. It shows three absorption-bands between D and F and is figured in fig. 18 (mounted in Balsam).

FIG. 18.—Antedonin, in balsam.

I have found hæmatoporphyrin and hæmatin in the following: Toxoponentes, Lividus, Sphærachinus, Echinus, Spatangus; pigments extractable by dilute acids. Those extractable by fat-solvents are probably lipochromes.

Moseley's Pentacrinin.—Numerous species of Pentacrinus of the deep sea contain this pigment which possesses a well-marked absorption-spectrum, consisting of three bands between D and F. Its other characters are referred to by von Fürth, and I have also found it in Pentacrinus.

The Aranidines.—The late Professor Krukenberg is responsible for this name.

The Pigments of Sponges.—These are mainly lipochromes, the floridines of Krukenberg, the Histohæmatins of the writer and spongioporphyrin, so named by Sir Ray Lankester. This latter occurs in Suberites Wilsonii and gives a very remarkable spectrum shown in the accompanying figure (Fig. 19); it is a unique pigment. Its absorption curve is also here shown in aqueous alkaline solution. The spectrum maps show it in aqueous and in acid alcohol solution. Lankester and I found spongioporphyrin most refractory towards solvents.

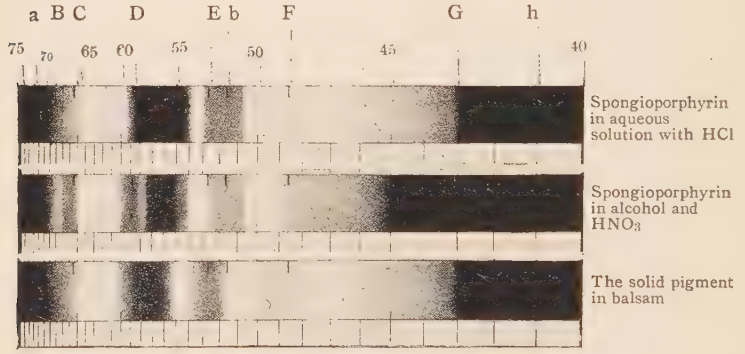

a B C D E b F G h
75 70 65 60 55 50 45 40
Spongioporphyrin in aqueous solution with HCl
Spongioporphyrin in alcohol and HNO_3
The solid pigment in balsam

FIG. 19.

Note.—The curve here shown is on a wave-length scale: that of Chart III. is not. The latter is not on the normal scale but is adapted to that of Zeiss's microspectroscope.

Pigments of Worms and Molluscs.—Sir Ray Lankester following other observers, e.g. Schunck and Sorby, examined the green pigment of Bonellia viridis which belongs to the Gephyreans. It is known as bonellin when dissolved in alcohol: it is purple and unchanged in colour by neutralization. It shows at least four bands between C and F. It was supposed to be

a chlorophyll, and is probably an immediate food-product like chætopterin (cf. Schenck).

Chætopterin.—This was discovered by Sir E. Ray Lankester. It occurs in the gut of this curious worm Chætopterus, which lives mainly on Diatoms and it is derived from the food eaten by the worm. The pigment is a dark green colour and fluoresces red like chlorophyll and has almost the same spectrum as the latter.

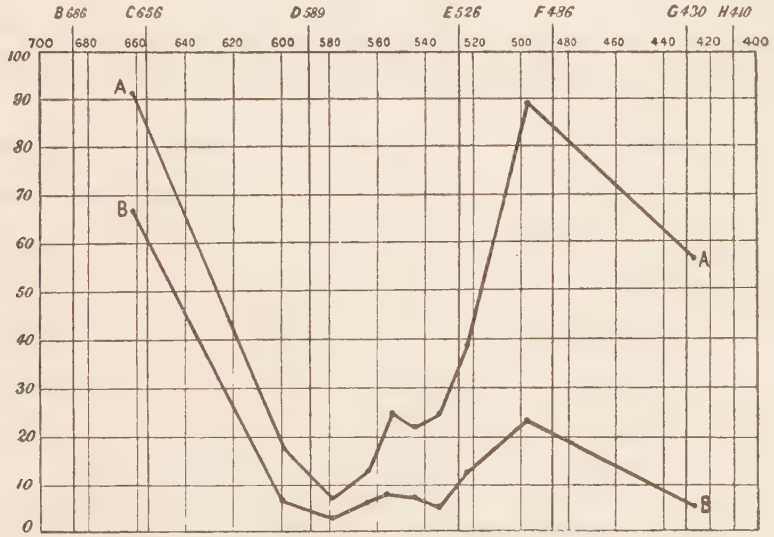


FIG. 19A.—Spectrophotometer curves of aqueous alkaline solutions of Sir Ray Lankester's spongioporphyrin.

Professor Engelmann examined this with his micro-spectrophotometer and figured it in Sir E. Ray Lankester's paper mentioned below (Fig. 20).

It is quite a different pigment in its spectrum from bonellin. Other green pigments, such as that of Phyllodoce viridis, Pentobdella, have been described by me. The other pigments of worms occurring in the integument have been as yet little explored.

The Pigments of Mollusca.—These fall under three headings thus: (1) Those belonging to the hæmatin series; (2) those like melanin; (3) those like pigments in the green oysters.

FIG. 20.—Oxychlorocruorin.

FIG. 20A.—Blood of serpula living animal.

Pigments of the Hæmatin Series (Fig. 21, 1-4).—Lankester found hæmoglobin in the pharyngeal muscles of many Gastropods, and showed that its presence appeared to be connected with the muscular activity of that part of the animal. I found hæmatoporphyrin, extractable by acidulated alcohol, in the

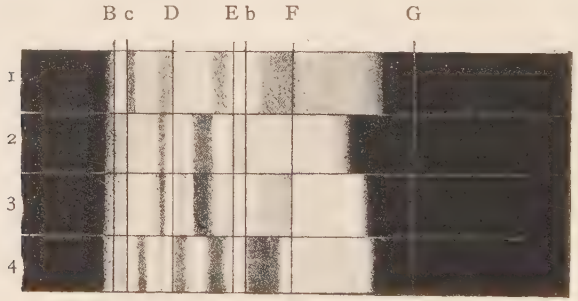


FIG. 21.

brown integument of species of Limax, of Arion and of Sollecurtus strigillatus. In was soluble in chloroform and spectroscopically identified as hæmatopor-

phyrin. Biliverdin has been found in the shells of Halictis, Turbo, and Trochus by extracting it with dilute mineral acid when a green pigment goes into solution. The late Professor Krukenberg identified it as biliverdin : it gives Gmelin's reaction. It is doubtful whether the integument of Limax does or does not contain hæmoglobin.

The pigments resembling melanin have no absorption-bands.

Aplysia possesses a pigment of an unknown kind which I isolated in an apparently pure condition. The brothers De Negri observed its spectrum and examined its properties. I do not propose to enter into the green colours of the oyster about which there has been so much discussion.

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